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Diaphragmatic blood flow at various levels of ventilation in the rabbit

CLAES AXÉN and PER OLOF JANSON
Department of Physiology University of Göteborg, Sweden

AXEN C. & JANSON P O Diaphragmatic blood flow at various levels of ventilation in the rabbit. Acta Physiol Scand 1979 106. 1 3 Received 22 August 1978. ISSN 0001 6772 Department of Physiology University of Göteborg, Sweden.

Diaphragmatic and renal blood flow were measured with Ytterbium-169 and Scandium-46 labelled 15 μm microspheres in sodium pentobarbitone anesthetized rabbits. The first measurement was performed during spontaneous breathing of air and the second measurement after 15 min of breathing 2–6% carbon dioxide in oxygen. The lung ventilation as well as the diaphragmatic blood flow increased significantly during breathing of the carbon dioxide–oxygen mixture Arterial blood pressure and renal blood flow were not significantly altered by the induced hyperventilation. No significant correlation was found between the magnitude of lung ventilation and diaphragmatic blood flow The results of the present study indicate that consecutive measurements of diaphragmatic blood flow with radioactive microspheres at various levels of breathing effort is an appropriate method for further exploration of the relationship between diaphragmatic perfusion and working performance.

Key words Diaphragmatic blood flow ventilation, rabbit, radioactive microspheres

It has been suggested that blood flow to the diaphragm is a limiting factor in maintaining respiratory work during heavy exercise (Tenney & Reese 1968). In agreement with this concept, Rochester & Pradel-Guena (1973) reported a positive correlation in anesthetized dogs between perfusion of the diaphragm and lung ventilation. The mean diaphragmatic blood flow under resting conditions reported by these authors using radioactive xenon clearance technique, was 42 ml/100 g min which is a value intermediary between those reported for skeletal muscle and myocardium. The relatively high level of diaphragmatic blood flow and the large scatter amongst values were suggested to be due to methodological factors, such as laparotomy and traumatic labelling It is therefore of interest to compare the magnitude of diaphragmatic blood flow as measured with xenon clearance to that measured with a less invasive technique which involves no mechanical interference with the diaphragm In the present communication blood flow to the diaphragm was studied at two levels of respiratory work in anesthetized rabbits using radioactive microsphere technique

MATERIALS AND METHODS

Animals Seven Swedish Land rabbits weighing between 2 and 4 kg were anesthetized with sodium pentobarbitone (Nembutal, Abbott, U.K., 30 mg/kg b w) and were tracheotomized. A Y-shaped tracheal cannula equipped with directional flow valves was inserted. The lung ventilation was measured with flow meter connected to the outlet valve of the tracheal cannula. Each animal was allowed to breathe air spontaneously for 10 min after which period the animals breathed either 2% CO_2 in 98% O_2 (=5) or 6% CO_2 in 94% O_2 (=?) in Douglas bag connected to the inlet valve of the tracheal cannula.

Microsphere technique Radioactive carbonized microspheres with diameter of 15±5 μm (range) were purchased from 3M CO (St Paul, Minn U.S.A) Two batches of spheres with separate labellings were used, namely ytterbium-169 (^{169}Yb) and scandium-46 (^{46}Sc) with initial specific activities of 8.79 and 10.33 mCi/g, respectively The spheres were suspended in 20% dextran. T inhibit aggregation of the spheres, tw drops of detergent (Teepol) were added to each batch. In each animal tw consecutive measurements of blood flow were made according to procedure described in detail previously (Janson 1975 Janson & Albrecht 1975) The first measurement using ^{169}Yb labelled spheres was performed after 10 min of spontaneous breathing of air and the second blood flow determination using ^{46}Sc labelled spheres after 15 min of breathing CO_2–O_2 mixture as described

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Table 1 *Diaphragmatic and renal blood flow in anaestheti_ed rabbits during spontaneous breathing of a and during breathing of CO_2–O_2 mixtures*

Animal no	Treatment	Mean arterial blood pressure (mmHg)	Lung ventilation (ml/min)	Bloow flow (ml/100 g min)					
				Diaphragm			Kidney		
				Right	Left	Total	Right	Left	Both
1	Air	104	901	81	64	71	590	620	605
	2% CO_2+98% O	106	1 316	90	70	79	448	505	473
2	Air	80	735	29	4	[illegible]6	358	359	346
	2% CO_2+98% O_2	78	909	3[illegible]	28	30	354	332	331
3	Air	84	1 220	70	68	69	457	46[illegible]	459
	2% CO_2+98% O_2	80	1 923	91	90	90	417	418	417
4	Air	115	733	56	57	56	588	617	604
	2% CO_2+98% O	112	1 111	59	60	59	650	677	652
5	Air	88	581	34	23	28	[illegible]69	299	284
	% CO_2+98% O_2	84	870	51	32	41	303	338	320
6	Air	88	541	26	[illegible]5	[illegible]5	498	533	494
	6% CO +94% O_2	88	1 333	62	6[illegible]	62	598	733	651
7	Air	78	909	61	46	54	503	493	498
	6% CO_2+94% O_2	78	1 471	83	72	78	453	463	460

above Blood flow per unit of weight was determined in the right and left muscular part of the diaphragm and in the right and left kidney

Statistical method Means and standard error of means were calculated according to conventional methods Paired comparisons were performed using Student s *t* test A *P* value of 0 05 or less was considered to be significant.

RESULTS

Methodological observations

The intracardiac injection of microspheres evoked no change in arterial blood pressure in any of the animals The mean number of microspheres en countered in the muscular part of the diaphragm allowed diaphragmatic blood flow to be measured with a mean precision of ±4 2% according to calcu lations presented by Buckberg et al (1971) The correlations found between blood flow per unit of weight of the right and left half of the diaphragm (0 97) and the right and left kidney (0 97) indicated an even distribution of microspheres

Lung ventilation and blood flow of diaphragm and kidney

Table 1 summarizes the values of lung ventilation and diaphragmatic and renal blood flow on two oc casions in each animal During spontaneous breath- ing of air the lung ventilation was 803±88 ml/min and it rose to 1276±192 ml/min during breathing of 2% CO +98% O_2 in 5 animals In two animals breathing 6% CO_2+94% O_2 the rise in lung ventila- tion was 109 and 62% respectively In 5 animals breathing air followed by 2% CO_2+98% O_2, dia- phragmatic blood flow rose from 47±8 to 60±11 ml/100 g min The corresponding rise in dia- phragmatic blood flow of the animals breathing air followed by 6% O_2+94% O_2 was 148 and 44% respectively

Paired comparisons in the group of animals breating air followed by 2% CO +98% O_2 showed significant increase in lung ventilation ($P \leq 0.02$) and in diaphragmatic blood flow ($P \leq 0.05$) Renal blood flow was not significantly changed

DISCUSSION

The present values for diaphragmatic blood flow at quiet breathing in rabbits are in good agreement with results previously reported in dogs using xe- non clearance technique (Rochester & Pradel-Guena 1973) Kety Schmidt technique (Rochester & Bettini 1976) and microsphere technique (Fixler et al 1976) The values are also in accordance with those obtained in the rabbit with indicator fraction- tion technique using rubidium (Mognoni et al 1974) The increase in diaphragmatic blood flow found during hyperventilation induced by CO_2 was

of the same magnitude as that reported by Mognoni et al. (1974).

The microsphere technique as used in the present study with two isotopes, permits blood flow to be determined at different levels of breathing effort in one and the same animal thus allowing the animal to act as its own control. As shown by Neutze et al. (1968) it is technically possible to make up to 4 consecutive injections of microspheres to rabbits without disturbing the hemodynamic situation. Blood flow of the respiratory muscles may thus be studied more than twice at various workloads. As only submaximal stimuli for hyperventulation were applied in the present study it cannot be determined whether blood flow is limiting factor for sustained high breathing efforts. An extension of the number of isotopes in measurements with microspheres might shed further light on this problem.

Using both clearance and indicator fractionation techniques, Rochester & Pradel-Guena (1973) and Mognoni et al. (1974) have reported higher maximal values for diaphragmatic blood flow than those found in the present study (108–146 ml/100 g min as compared to 90 ml/100 g min). The most probable reason for this difference may be sought in the fact that the increase in lung ventilation of the animals of the present study was moderate. On the other hand values obtained with clearance techniques are difficult to interpret in hyperperfused tissues due to very steep initial parts of the clearance curves. Calculation of flow from such curves very often lead to underestimations. At high blood flow values also indicator fractionation methods may lead to underestimation of flow due to an incomplete extraction of the tracer from blood vessels to tissues (Janson & Albrecht 1975). It may thus be preferable to use radioactive microspheres in studies where diaphragmatic blood flow is expected to be very high for instance at high respiratory workloads.

Financial support was obtained from The Medical Faculties of Lund and Goteborg.

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	2% CO_2+98% O_2	78	909	32	28	30	354	332	331
3	Air	84	1 220	70	68	69	457	462	459
	2% CO_2+98% O_2	80	1 923	91	90	90	417	418	417
4	Air	115	733	56	57	56	588	617	604
	% CO +98% O	112	1 111	59	60	59	650	677	652
5	Air	88	581	34	23	28	269	299	284
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6	Air	88	541	26	25	25	498	533	494
	6% CO_2+94% O_2	88	1 333	62	62	62	598	733	651
7	Air	78	909	61	46	54	503	493	498
	6% CO_2+94% O_2	78	1 471	83	72	78	453	463	464

above Blood flow per unit of weight was determined in the right and left muscular part of the diaphragm and in the right and left kidney

Statistical method Means and standard error of means were calculated according to conventional methods Paired comparisons were performed using Student's *t* test A *P* value of 0 05 or less was considered to be significant.

RESULTS

Methodological observations

The intracardiac injection of microspheres evoked no change in arterial blood pressure in any of the animals The mean number of microspheres en countered in the muscular part of the diaphragm allowed diaphragmatic blood flow to be measured with a mean precision of ±4.2% according to calcu lations presented by Buckberg et al (1971) The correlations found between blood flow per unit of weight of the right and left half of the diaphragm (0 97) and the right and left kidney (0 97) indicated an even distribution of microspheres

Lung ventilation and blood flow of diaphragm and kidney

Table 1 summarizes the values of lung ventilation and diaphragmatic and renal blood flow on two oc casions in each anima During spontaneous breath- ing of air the lung ventilation was 803±88 ml/min and it rose to 1226±192 ml/min during breathing of 2% CO_2+98% O_2 in 5 animals In two animals breathing 6% CO_2+94% O_2 the rise in lung ventila- tion was 109 and 62% respectively In 5 animals breathing air followed by 2% CO +98% O_2 dia- phragmatic blood flow rose from 47±8 to 60±11 ml/100 g min The corresponding rise in dia- phragmatic blood flow of the animals breathing air followed by 6% O +94% O_2 was 148 and 44% respectively

Paired comparisons in the group of animals breating air followed by 2% CO_2+98% O_2 showed significant increase in lung ventilation ($P \leq 0.02$) and in diaphragmatic blood flow ($P \leq 0.05$) Renal blood flow was not significantly changed

DISCUSSION

The present values for diaphragmatic blood flow at quiet breathing in rabbits are in good agreement with results previously reported in dogs using xe- non clearance technique (Rochester & Pradel-Guena 1973) Kety Schmidt technique (Rochester & Bettini 1976) and microsphere technique (Fixler et al 1976) The values are also in accordance with those obtained in the rabbit with indicator fraction- tion technique using rubidium (Mognoni et al. 1974) The increase in diaphragmatic blood flow found during hyperventilation induced by CO_2 was

Continuous measurement of flow rate and volume in the nanoliter range

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A simple transducer which is based on changes of electrical capacity with volume is described. The system accomodates fluid volumes of several microliters and the resolution is below one nanoliter The response time of the transducer is about 10 ms. The system is applicable for continuous recording of flow rates in renal collecting tubules. Pulsatile flow in the duct of Bellini is demonstrated.

Key words Nanoliter measurement, volume transducer collecting duct, rat kidney

Determination of the flow rate in a renal tubule is usually performed by quantitative collection of tubular fluid over a known period The amount of collected fluid can be determined by transference to a constant bore glass capillary and measuring the length of the fluid column (Ullrich et al 1969) Alternatively the diameter of the drop formed spontaneously under mineral oil can be measured (Hellman et al. 1967)

Elaborate systems for more rapid recording of the collection rate have been worked out. Determination of the filling time of a calibrated glass capillary cemented into the tip of the collection pipette gives a rapid estimation of the flow (Seiller & Gertz 1977) Other systems are based upon continuous aspiration by a micropump The pressure in the collection system is kept constant by adjusting the speed of the pump (Hierholzer et al. 1972) The flow rate is recorded from the pump setting. Another system operates to keep a constant position of a fluid meniscus in a side arm of the collection system by feed-back activation of a step pump (Fishbarg et al 1977)

A more direct approach to measurement of the flow rate is obtained by a capacitative recording of the movement of the fluid meniscus in the collection system (Wiedner 1976) A similar system has recently been constructed in this laboratory and was found to work excellently even under conditions of rapidly changing flow rates.

MATERIAL AND METHODS

The volume transducer A steel cannula (gauge 27) is cemented together with the end of a constant bore glass capillary (0.334±0.002 mm ID, Corning, USA) by dental wax (Fig. 1). The capillary is cemented into a hypodermic needle which in turn is supported by a mantle of glass covered by rubber The constant bore glass capillary is filled with mercury from the luer fitting at the end of the hypodermic needle (Fig. 1). The steel cannula is then inserted into an oil filled collection pipette This connection is secured with dental wax. The hypodermic needle is connected to pressure control system (Gaeter manometer) by air filled nylon tubing. This permits control of the movement of the mercury column in the constant bore capillary The mercury is pushed into the end of the collection pipette displacing some of the oil through the tip by applying pressure to the Gaeter manometer The system is connected electrically by attaching metal clamp to the steel cannula and another to the hypodermic needle.

The electronic recording system. Volume flow into the transducer displaces the mercury into the constant bore glass capillary and causes change in capacitance between the hypodermic needle and the mercury column in the capillary This change is measured by reactance converter (type 51E01 DISA, Denmark) designed for capacitative transducers. Thus the system is based on frequency modulation of carrier wave by the change in capacitance. This change in turn is converted to DC voltage which follows the modulation of the oscillator frequency The reactance converter can be tuned to the oscillator frequency by turning dial from which the "zero frequency" i.e. the frequency at which the output from the converter is zero can be read. The zero frequency is dependent on the capacitance of the transducer and it can be varied between 4.5 MHz and 5.5 MHz which permits the system to be used over a considerable range of

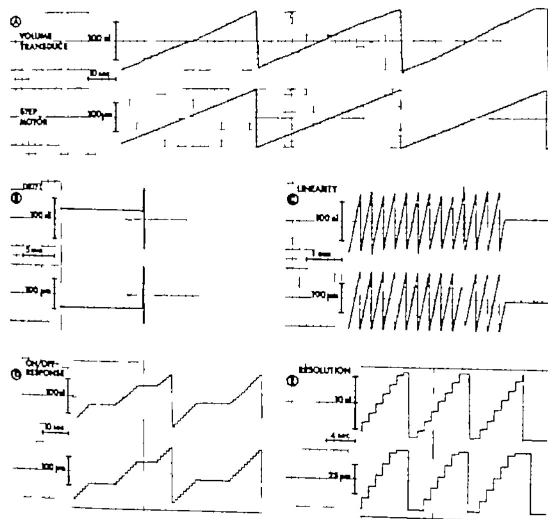


Fig 2 Output curves from the volume transducer and the step micromanipulator on the upper and lower traces re spectively The downstrokes are caused by manual resetting of the electronics in the tw systems. In A–D 50 µl Hamilton syringe is used and connected to the collection pipette by oil filled nylon tubing. I E a 10 µl Unimetrics syringe is connected directly to the volume transducer

from the pressure pipette Furthermore back leakage of fluid from the surface of the papilla was excluded by notion that lissamin green applied to the surface did not enter the collection ystem.

RESULTS AND DISCUSSION

Fig. 2 shows the responses to a stepwise filling of the glass capillary and the movement of the step micromanipulator on the upper and lower traces, respectively When the upper limits of the pen movements were reached the systems were reset manually by decreasing the zero frequency of the reactance converter slightly and pressing the reset button for the read out from the step motor The speed of the paper is calculated from the frequency of the step motor and the inclinations (α) of the curves are measured by a goniometer and the slopes expressed as tgα.

Fig. 2 A shows 3 typical calibration curves. The flow rate delivered by the microsyringe was 196 nl min as calculated from the speed of the piston The standard deviation (S.D) of the mean value for tgα of 9 consecutive calibration curves (3 of them

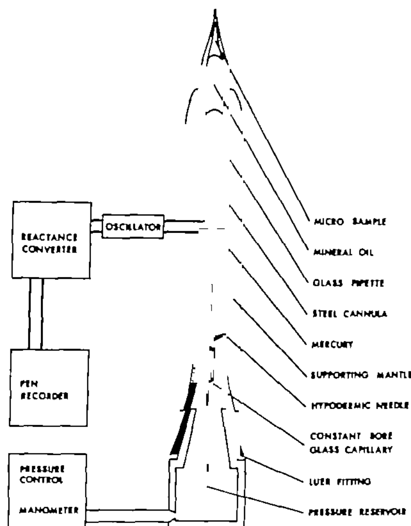


Fig. 1. The volume transducer. For details see description in method's section.

capacitance. The sensitivity of the assembled system is about 20 mV nl⁻¹.

The mechanical test system. For daily calibration of the collection system a 50 μl syringe mounted on a Sage pump (model 341, Sage Instruments, USA) is utilized and connected to the collection pipette via nylon tubing filled with mineral oil. The pressure control system is set at zero, i.e. atmospheric pressure. Furthermore a critical test of the system was done by means of a step micromanipulator (Eide & Källström 1968) with variable step length and frequency (manufactured at our institute). A stepmotor (Philips PD 12) is utilized with a gear ratio of 125: 24. The rise time is 1.5 ms for a single step of μm. The step micromanipulator was mounted to advance the piston of a 10 μl or a 50 μl syringe connected to the volume transducer. The control unit for the step motor gives a DC voltage read out of the travelled distance of the piston which is fed into one channel of a pen recorder (Brush 220). This DC output can be reset manually without interfering with the movement of the piston.

The biological preparation. Young Wistar rats weighing between 80 g and 100 g were anesthetized by Inactin (120 mg per kg b.wt. i.p.) and placed on a servocontrolled heating pad keeping the body temperature at 37.5°C (Anitemp, ATEW, Sweden).

A tracheostomy was performed. An indwelling catheter was placed in the right jugular vein for infusion of 0.9% NaCl at a rate of 2.25 ml per hour administered by a Braun pump (Germany). The left kidney was prepared for micropuncture and immobilized in a lucite cup covered by mineral oil prewarmed to 37°C. The papilla was exposed by gentle traction of the ureter and the pelvis was cut with a fine pair of scissors (Ullrich et al. 1961). This procedure left the terminal end of the papilla free. A Bellini duct was punctured by a pressure measuring pipette at a distance from the pelvic end of the duct about 250 μm. A servo nulling system (Wiederhielm et al. 1964) was used for pressure measurements employing 3 μm pipettes filled by 1 M NaCl 1% lissamin green. An oilfilled collection pipette with an inner tip diameter of about 20 μm was introduced axially about 50 μm into the tip of the Bellini duct in a wedged position to give a tight fit between the duct and the pipette. The tubular fluid was collected at the control free flow pressure by adjusting the pressure in the Gauer manometer. To secure that no leakage occurred past the collection pipette occasionally a small bolus was injected

Jorthe Davidsen and Svend Christoffersen provided technical assistance

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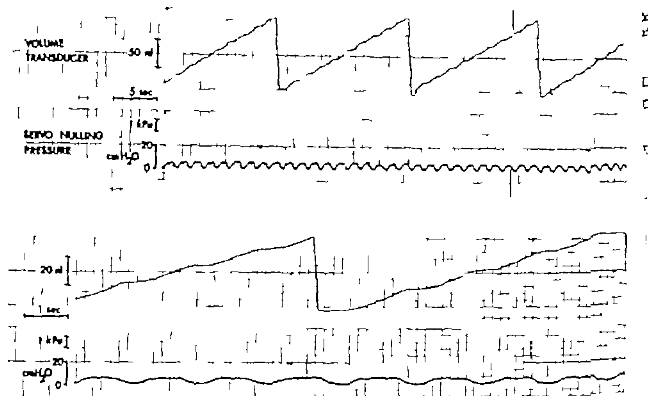


Fig 3 The upper panel shows the aspirated volume and the hydraulic pressure in the terminal collecting duct (Bel- *lini duct) on the upper and lower trace respectively* The lower panel shows the same events at higher amplification and paper speed The downstrokes on the volume curve are caused by manual resetting of the zero frequency of *the reactance converter*

being shown in Fig 2 A) are 5 7% and 0 7% of the mean values for the upper and lower traces respectively Fig 2B shows the stability of the base line *The deflections in the middle of the run was caused* by a short activation of the stepmotor The maximum zero drift is about 0 4 nl min Fig 2C shows the linearity of the system The repeated resettings changed the zero frequency of the reactance converter from about *5 MHz* to *4 8 MHz* The standard deviation (S D) is 3.3% of the mean value of the slopes of the volume curves ($n = 13$) Trend analysis of the successive slopes yields a correlation coefficient ($r = -0$ 23 $P > 0$ 4) which indicates that they do not change significantly due to the repeated resettings Accordingly the sensitivity is constant over a large range of *volumes Fig 2 D* shows the effect of 3 consecutive stoppings of the step motor The volume curves closely follows the movement of the piston Fig. 2 E shows the resolution of the system Each step of the manipulator is 10 µm with a rise time of 7.5 ms This corresponds to a volume displacement of 1 9 nl (S D = 0 1 nl $n = 16$) with a response *time* about 10 ms

Recordings during a total collection of fluid from the terminal collecting duct of the exposed renal papilla are shown in Fig. 3 The upper and lower *traces show the cumulated fluid volume* in the collection system and the pressure in the terminal collecting duct respectively The mean flow rate recorded from the slope of the volume curves is [illegible] nl min and the corresponding driving pressure was 5.5 cmH_2O (0.54 kPa) This gives a resistance of 1 kPa min μl^{-} indicating that the resistance of *the Bellini duct is high in comparison* with those of the upstream parts of the tubular system (Kriz hoffer 1960 Marsh & Martin 1975)

Pulsations in flow and pressure occur at a frequency of 39 min i e the frequency of the pelvic *contractions (Schmidt Nielsen 1977) The* pressure varies between 1 5 cmH O and 7.5 cmH_2O (0 15 kPa and 0 74 kPa) The flow varies by a factor six in phase with the hydraulic pressure pulsations. This is in accordance with observations made in the *hamster (Steinhausen 1964)* and strong evidence for a pelvic milking of the renal papilla in spite of the exposure of the tip

vidence for a cholinergic secretory innervation f the guinea pig endometrium

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HAMMARSTRÖM, M & SJÖSTRAND N O Evidence for a cholinergic secretory innervation of the guinea-pig endometrium. Acta Physiol Scand 1979 106: 11–15 Received 7 Sept. 1978. ISSN 0001-6772. Department of Physiology Karolinska Inst., Stockholm, Sweden.

Uterine mucus secretion was studied by measuring carbohydrate release from everted guinea-pig uteri placed in organ baths. Field stimulation of the organ resulted in significant increase in carbohydrate out-put, this increase was inhibited by tetrodotoxin, scopolamine and previous destruction of the paracervical ganglia. Previous section of the hypogastric nerve reduced the response to field stimulation, while interruption of the costouterine connexion or the pelvic nerve was without overt effect. Stimulation of the hypogastric nerve increased uterine carbohydrate secretion. Pelvic nerve stimulation produced no significant effect. Carbachol increased the carbohydrate secretion to the same extent as did field stimulation. Isoprenaline and methoxamine were without significant effect. It is suggested that certain endometrial cells have a cholinergic sympathetic secretory innervation with pre- and postganglionic fibers running in the hypogastric nerve.

Key words Uterus, endometrium, cholinergic nerves, mucus secretion, autonomic nerves

The innervation of the mammalian uterus has been the subject of a rather intense interest since the eighteenth century (vide e.g. Lee 1841 1842 1858 Frankenhäuser 1867 Langley & Anderson 1896 Dahl 1974 Gruber 1933 Jabonero 1953 Krantz 1959 Bell 1974 Owman, Sjöberg & Sjöstrand 1974 Thorbert et al 1977) However from a physiological point of view this interest has mainly been focused on myomotor nerves, action of neurotransmitters on the myometrium, changes in transmitter content of the adrenergic nerves apparently supplying the uterine smooth muscle and on the innervation of the uterine vessels Astonishingly little interest has been paid to the innervation of the endometrium

Thus, despite the attention given to endometrial function as far as we know no physiological study has been performed on efferent endometrial innervation though nerves to the secretory linings have been described in several morphological studies (Kilian 1841 Frankenhäuser 1867 Patenko 1880 Gawronsky 1894 Dahl 1974 Davis 1933 Brown & Hirsch 1941 State & Hirsch 1941 Koppen 1950, Jacobson & Nieves 1961 Casaglia & Giro 1963 Coupland 1962, 1968 Adham & Schenk 1969 Dallenbach & Vonderlin 1973 Kühnel & Beier 1976) Secretomotor function of these nerves has been proposed (e.g. Dallenbach & Vonderlin 1973 Kühnel & Beier 1976) but has seemingly never been proved or experimentally documented This was the aim of the present study

In this report an in vitro method for the study of uterine secretion is described. Its basis is the wellknown fact that uterine mucus contains large amount of carbohydrate which accordingly can be used as marker of endometrial secretion. We will present data indicating that the endometrium has cholinergic secretory innervation and that secretory cells respond to muscarinic drugs. Furthermore we will attempt to shed light on the possible pathways of such secretory nerves.

MATERIAL AND METHODS

Animals Mottled or albino virgin guinea-pigs (300–350 g) treated with estradiol benzoate 6 mg/kg and hydroxyprogesterone caproate 150 mg/kg (Primosiston® Schering AB) every second day for 10–14 days, in order to bring the endometrium into secretory phase, were used.

Dissecting and mounting procedures The animals were

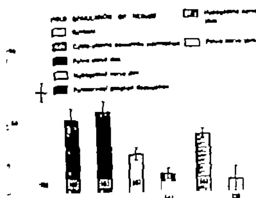


Fig. 2. Effects of various denervations on carbohydrate secretion of guinea-pig uterus in response to field stimulation as well as effect of hypogastric and pelvic nerve stimulation on carbohydrate secretion. Note the decrease following hypogastric denervation as well as destruction of paracervical ganglia. Number of experiments within brackets (mean ± S.E.)

related to uterine tissue the average secretion corresponded to 0.68±0.30 μmol of glucose/g wet weight. (Mean ± S.D. n=48). All values are uncorrected for losses due to extraction.

Field stimulation (Figs. 1 and 2).

Field stimulation at the standard rate of 0.5 Hz yielded an increase in secretion of carbohydrate by about 70% of resting level. Higher stimulation rates did not produce any further increase. The tendency rather was that of diminished secretion.

Pharmacology. The increase in carbohydrate secretion produced by field stimulation was abolished by tetrodotoxin (TTX) ($3\times10^{-}$ M) and scopolamine (3×10^{-6} M) while it was left unimpaired by the adrenergic blockers phentolamine (1.6×10^{-6} M) and propranolol ($1.8\times10^{-}$ M) (Fig. 1).

Denervation experiments. No significant reduction in secretion evoked by field stimulation was observed after interruption and degeneration of the pelvic nerve or the nerves of the costouterine connection. However, section and degeneration of the hypogastric nerve reduced the evoked carbohydrate secretion to about one-third ($P<0.001$) of the control level. Destruction of the paracervical ganglia produced a further decrease to one sixth of the control level ($P<0.001$) (Fig. 2).

Stimulation of hypogastric and pelvic nerves (Fig. 2)

No certain increase in carbohydrate secretion above resting level was obtained by pelvic nerve stimulation. On the contrary stimulation of the hypogastric nerve produced a definite increase (+40%) in carbohydrate secretion.

Effects of carbachol, methoxamine and isoprenaline (Fig. 3)

Carbachol raised the carbohydrate secretion to the same extent as did field stimulation. The effect was characterized by a rather sharp threshold at about 5.5×10^{-8} M whereafter maximum response rapidly occurred. The effect of $5.5\times10^{-}$ M of carbachol was abolished by scopolamine (1.3×10^{-6} M).

The α-receptor stimulant methoxamine (4×10^{-6} M or 4×10^{-4} M) and the β-receptor stimulant isoprenaline (2×10^{-6} M or 2×10^{-6} M) were without overt effect on the secretion.

DISCUSSION

Before interpreting our results some critical comments on the method ought to be mentioned. The method is rough and does not permit any sophisticated analyses. For instance: (a) The chemical determination is merely a test for total carbohydrate content. (b) The condition of the everted uterus must be far from natural. (c) The sampling periods were performed in glucose-free medium, otherwise the glucose of the Tyrode solution would have jeopardized the carbohydrate determinations. However, although due precaution should be taken with respect to transferring our results to the in vivo secretion of uterine mucus, we believe that the following conclusions are valid:

1. Certain uterine cells responsible for carbohydrate secretion seem to have excitatory muscarinic receptors as evidenced by the effect of carbachol. In this context it should be recalled that pilocarpine has been reported to alter the dynamics of human cervical mucus secretion (Kaulla et al. 1957).

2. Certain uterine secretory cells seem to have an excitatory innervation as revealed by the TTX susceptible response to field stimulation and the virtual absence of effect of field stimulation after phenolization of the paracervical ganglia through which most efferent uterine nerves in the guinea-pig seem to pass (vide e.g. Bell 1972, Thorbert et al. 1977).

3. This secretory innervation seems to be cholinergic since scopolamine abolished the effect of field stimulation. This would be in accordance with the

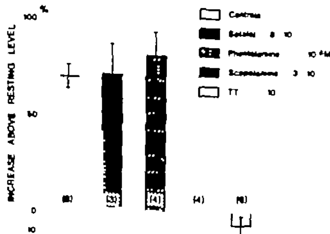


Fig 1 Influence of drugs on carbohydrate secretion of guinea-pig uteri in response to field stimulation (0.5 Hz, 0 1 ms 30 V 10 min) The secretion is left unimpaired by sotalol and phentolamine but is abolished by scopolamine and TTX $*P<0\ 001$ (different from controls) Number of experiments within brackets (mean±S E)

stunned and bled the uterus and parametrial tissue were dissected out Great care was taken to prepare the uterus free from all vaginal structures. Each horn including its part of the cervix was then prepared free from adjacent structures the tubal end of the horn was ligated invaginated and the everted horn mounted on a platinum electrode for field stimulation (vide Sjöstrand & Swedin 1970) The organ was put into a 25 ml bath in which another electrode was placed the bath contained Tyrode solution aerated with 6 5% CO_2 in O_2 and kept at a temperature of 36°C

A modified procedure was performed when the pelvic or hypogastric nerves were stimulated The uterus and parametrial tissue together with the hypogastric or pelvic nerve were dissected out The horn was sectioned and opened through the medial wall turned inside out and fixed on a polyethylene catheter before being placed into the organ bath. The nerves were placed within the bath on platinum electrodes (2 mm apart) at a distance of 1–2 cm from the uterus

Nerve stimulation parameters Field stimulation was applied throughout the sampling periods (see below) with rectangular pulses of 0.5 ms duration 30 V and of a standard frequency of 0.5 Hz. Stimulation of hypogastric and pelvic nerve was applied with a frequency of 5 Hz (30 V 0.1 ms) for 10 s every 30 s during the sampling period The pulses were delivered from Grass S44 or SD 9 stimulators

Operative procedures Bilateral denervation was performed under sodium pentobarbital anesthesia (30–40 mg/kg) (when necessary supplemented with ether) 10–12 days before an experiment was undertaken. About 2 mm of each *hypogastric nerve* was removed 1 cm below the inferior mesenteric ganglion Each *pelvic nerve* was similarly sectioned 1 cm proximal to its crossing of the iliac artery The *paracervical ganglia* were destroyed with phenol as described by Bell (1974). The *nerves* of the *costo-uterine connexions* (Gabella 1976, Thorbert et al. 1977) were destroyed by tight ligature with a silk [illegible] dipped in 8.5 m phenol

Estimation of uterine carbohydrate secretion The [illegible]eral procedure was as follows. The organs were [illegible]dated for 80 min in ordinary Tyrode solution, [illegible] every 20 min. Thereafter each horn was used for 5 [illegible]pling periods of 10 min during which the organ [illegible] bathed in a Tyrode solution in which the glucose [illegible] replaced by NaCl Between each collecting period [illegible] was an interlude of 20 min in ordinary Tyrode solution The first third and fifth sampling periods were [illegible] determination of the resting secretion level. During [illegible] second and fourth collecting periods the organ was [illegible]posed to drugs and/or nerve stimulation In order to [illegible] contamination with glucose from the ordinary Tyrode [illegible]ution, the bath and the organ were carefully rinsed [illegible] glucose-free Tyrode solution twice before a collecting period started

The collected fluid from the sampling periods (25 [illegible] was analysed with a modification of the sulfuric acid [illegible]dole method of Dishe & Popper 1926 (vide Shettles [illegible] 1951 Shettles 1951) The fluid was lyophilized and the residuum dissolved in 0.5 ml distilled water and 4.5 [illegible] 14 4 M H_2SO was added After centrifugation 3 ml of the supernatant was removed and 0 1 ml 8.5×10^{-3} M [illegible] was added and the sample was heated to 100°C for [illegible] The samples were analysed at 470 nm on a Beckman Spectrophotometer B The amounts were calculated [illegible] glucose using an internal standard Certain samples [illegible] also checked against an internal standard of galactose This gave similar differences between the values of [illegible] and experimental periods as did the glucose standard Recovery of added glucose (2×10^{-7} $5\times10^{-}$ $7\times10^{-}$ 1×10^{-6} mol) yielded 96.5±9.5 (M±S D n=8).

Calculation and statistics Because of rather great [illegible]ance between different experiments in resting level [illegible] carbohydrate secretion all results were calculated as [illegible] cent of resting secretion using the mean of the three [illegible]ing periods as matching value Each type of experiment was performed on uteri from at least 3 animals.

The Student s *t*-test was used for the statistical [illegible]parison of experiments

Drugs Scopolamine hydrobromide (Sigma), phentolamine chloride (CIBA) sotalol hydrochloride (M[illegible] Regis Chemical Company) carbamylcholine chloride (Carbachol) (Sigma) Isoprenaline sulphate (Abbott) methoxamine hydrochloride (BDH) tetrodotoxin (TT[illegible]) (Sigma)

Tyrode solution composition NaCl $13\ 7\times10^{-2}$ KCl $2\ 7\times10^{-}$ $MgCl_2$ $4\ 9\times10^{-4}$ $CaCl_2$ 1.8×10^{-3} $NaHCO_3$ 1.2×10^{-2} Na_2HPO $2H_2O$ $3\ 1\times10^{-4}$ M $1\ 1\times10^{-2}$ mol [illegible] glucose was added per litre of ordinary Tyrode solution and $5\ 6\times10^{-}$ mol of NaCl to each litre of glucose-free Tyrode solution.

RESULTS

Resting secretion

The resting secretion of the uterus preparation corresponded to 0 13–0 78 µmol of glucose/10 min or 0.16–1 1 µmol of galactose/10 min respectively

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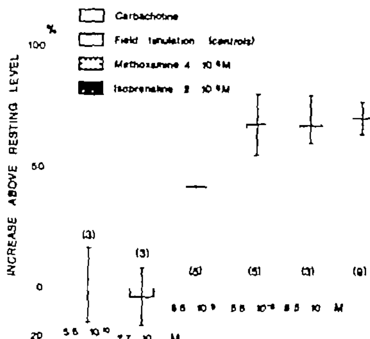


Fig 3 Effect of carbachol methoxamine and isoprenaline on the uterine carbohydrate secretion Carbachol increases the secretion to the same extent as does field stimulation while methoxamine and isoprenalme do not increase it Note the steepness of the dose response relationship to carbachol Number of experiments within brackets (mean ± S E)

work of Coupland (196? 1968) and Adham & Schenk (1969) demonstrating acetylcholine esterase positive nerves in the human and in the rat uterine mucosa. Such nerves have however hitherto not been described in guinea-pig endometrium although there are nerves (Jacobson & Nieves 1961)

4 The external pathway of the secretory nerves seems to be the hypogastric nerve The denervation experiments indicate that the hypogastric fibers are partly postganglionic partly preganglionic the latter relaying in the paracervical ganglia. Thus this secretory cholinergic innervation appears to have a similar pattern with long and short neurons as the myometrial adrenergic innervation (e.g. Owman Sjöberg & Sjöstrand 1974 Thorbert et al 1977) and might be classified as sympathetic cholinergic fibers e g the sudomotorfibers in certain species (Langley 1891 Sjöquist 1963) We observed no clear cut evidence for secretomotor fibers travelling in the pelvic nerve or in the costouterine connexion The roughness of the method makes it however impossible to definitely rule out the possibility that certain secretory nerves may reach the uterus via these connexions Also the possible existence of adrenergic inhibitory or stimulatory receptors on the secretory cells cannot be excluded although we obtained no positive evidence for any significant adrenergic mechanisms in this system

The secretory response to carbachol as well as nerve stimulation showed little gradation, it almost of all or none type This may reflect biological features of the response and could be due to a rapidly obtained high degree of occupancy of a relatively restricted number of muscarinic receptors on secretory cells and/or to the secretory process which may be of the apocrine type (vide e g. Hafez Ludwig & Metzger 1975) It could however also be due to an experimental artefact e g defective nourishment of axons and secretory cells during the collecting periods

The present results emphasise that nervous as well as humoral mechanisms should be considered in the control of uterine mucus secretion and that such nervous mechanisms may have a biological significance

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Inhibition of renal sympathetic nerve traffic from cardiac receptors in normotensive and spontaneously hypertensive rats

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RICKSTEN S E. NORESSON E. & THORÉN P Inhibition of renal sympathetic nerve traffic from cardiac receptors in normotensive and spontaneously hypertensive rats Acta Physiol Scand 1979 106: 17–22. Received 7 Sept. 1978 ISSN 0001-6772. Department of Physiology University of Göteborg, Sweden.

The reflex inhibition of the sympathetic outflow to the kidney was examined during volume load with horse plasma in 6 normotensive rats (NCR) and 6 spontaneously hypertensive rats (SHR). The rats were anesthetized with chloralose and urethane The arterial baroreceptors were denervated. The renal nervous inhibition was mediated via the vagal nerves and was mainly due to activation of receptors in the left side of the heart. The average thresholds in mean left atrial pressure for renal nervous inhibition was 5.4 mmHg for NCR and 9.2 mmHg for SHR indicating clear resetting of the reflex arch in the hypertensive animal: The reason is probably decreased distensibility of the wall of the left atrium due to chronic levation of left atrial pressure. This resetting of the atrial receptors in the hypertensive animals is probably of importance to allow an adequate filling pressure of the hypertrophied left ventricle and might also be of importance for the reflex neural control of renal function in these animals.

Key words Cardiac receptors, spontaneously hypertensive rats, resetting

Resetting of arterial baroreceptors in hypertension was first reported in the renal hypertensive dog by McCubbin et al (1956) and Kezdi (196.) This phenomenon has more recently been examined in the spontaneously hypertensive rat (SHR) Thus, Nosaka & Wang (197') and Jones (1977) reported significantly higher carotid sinus threshold pressures in order to elicit systemic hypotensive responses in SHR compared to normotensive control rats (NCR)

However there are also different receptor populations in the cardiopulmonary region with afferents travelling in the vagal nerves One type of endings mainly located at the right and left vein-atrial junctions (Paintal 1973) and these receptors are subserved by medullated afferent fibres. The large population of endings with non-medullated afferent fibers is more spread throughout the entire cardiopulmonary area both in the atria (Coleridge et al 1973 Thorén 1976) pulmonary veins (Coleridge & Coleridge 1977) and in the left ventricle (Coleridge et al. 1964 Sleight & Widdicombe 1965 Öberg & Thorén 1972 Thorén 1977) These receptors exert a tonic inhibitory influence on the resistance and capacitance vessels via the vasomotor center (Thorén et al 1976) Reflexes from cardiopulmonary afferents show a somewhat different efferent pattern in comparison with the arterial baroreceptor reflexes Thus the former have a relatively greater effect on the renal vessels than the muscle vessels

So far no studies have been performed upon the reflex effects elicited by the cardiopulmonary receptors during arterial hypertension. The aim of the present study was therefore to examine the inhibition of the sympathetic nervous activity to the kidney from the cardiac receptors in the SHR in comparison to the normotensive control rats (NCR)

METHODS

Seven male spontaneously hypertensive rats (SHR) and 11 normotensive control rats (NCR) both of the Wistar strain

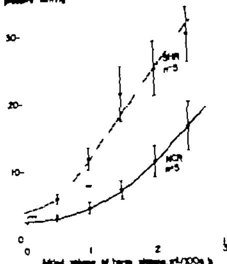


Fig. 3 A. The relationship between the transfused volume of horse plasma in ml/100 g b w. and the changes in mean left atrial pressure for 5 SHR and 5 NCR (*$P<0.05$ **$P<0.01$ ***$P<0.001$)

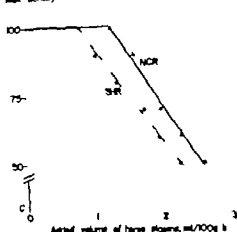


Fig. 3 B. The relationship between the transfused volume of horse plasma in ml/100 g b w. and the inhibition of mean rectified renal nerve traffic expressed as per cent of maximal control activity

some expts. the rate of the infusion was increased to 3.5 ml/min at the end of the expts. I 5 of the expts. the vagal nerves were cut when the rats were maximally plasma loaded. In 3 baroreceptor denervated rats cuffs were put around the ascending aorta and the pulmonary trunk. The pressure was selectively raised in the left and the right heart by occlusion of the aorta and the pulmonary artery. Arterial blood pressure, left atrial pressure and renal nervous activity were continuously registered. In all expts. the postmortal nervous activity was registered for 30 min to achieve the noise activity and which was 1-2 μV. The differences between the SHR and the NCR were compared using group comparison *t*-test. Values below 0.05 were considered as significant.

RESULTS

I. Renal nerve traffic and left atrial pressure. Fig. 1 is a recording from a typical expt. in a normotensive rat (NCR). Mean left atrial pressure before the start of infusion was significantly different: 4.?±0.6 mmHg in SHR and 2.7±0.8 in NCR ($P<0.001$). The constant infusion of horse plasma induces a prompt increase of the arterial blood pressure which levels off after about 1 min. Due to an effective denervation of the baroreceptors the renal nervous activity was not affected by this increase in arterial blood pressure. Following the horse plasma infusion mean left atrial pressure increased progressively and at a rather distinct pressure (in this expt. 5-6 mmHg) renal nervous activity started to be inhibited. Upon further transfusion there is a progressive decrease in renal nerve traffic. The relation between the mean left atrial pressure and the renal nervous activity (expressed as per cent of control activity) was plotted in 12 expts. Mean values for the two groups (6 SHR and 6 NCR) are plotted in Fig. 2. The mean left atrial "threshold" pressure is nearly twice as high in SHR (9.2±1.3 mmHg) than in NCR (5.4±0.7 mmHg) ($P<0.001$). The curve is also steeper for NCR.

Renal nerve traffic and changes in plasma volume. The curve relating the increase in plasma volume per 100 g b wt. and the average increase in mean left atrial pressure was constructed in 10 expts. (5 NCR, 5 SHR). For a given increase in plasma volume per 100 g b wt. the mean left atrial pressure increased more for SHR than for NCR both relative to the preinfusion pressure and in absolute terms (Fig. 3 A).

From the data presented in Fig. 2 and 3 A the relation between the added plasma volume (ml/100 g b.wt.) and the inhibition of renal nervous activity was constructed. A smaller volume per 100 g b wt. is needed to reach the threshold pressure of the left atrium for nervous inhibition in SHR compared to NCR (Fig. 3 B).

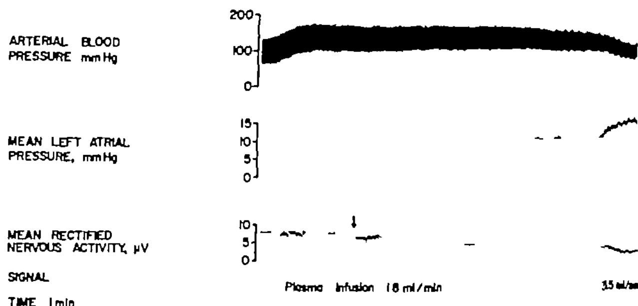


Fig 1 Effect of the horse plasma infusion on aterial blood pressure mean left atrial pressure and mean rectified renal nervous activity in a normotensive rat. The horse plasma was first infused at a rate of 1.8 ml/min and later with 3.5 ml/min The arrow indicates the first sign of reflex inhibition of renal nerve activity The left atrial pressure at this point is called the threshold pressure for the reflex

(Mollegaard breeding laboratory Denmark) were used in this study The average body weight of the SHR was 385 g ± 20 for the NCR 326±46 g both being at the age of 3–5 months and the SHR somewhat older than the NCR. The awake blood pressure was measured i a in the caudal artery *after light ether anesthesia. The* mean blood pressure was 155±8 mmHg for the SHR and 107±8 mmHg for the NCR ($P<0.001$) The rats were then anesthetized with urethane (500 mg/kg) and chloralose (50–100 mg/kg) i.a. and the rats were tracheotomized The carotid sinus baroreceptors were denervated bilaterally by coating the sinus with a solution of 10% phenol in ethanol which destroys the nerve endings of the sinus nerve (Krieger 1964) The aortic baroreceptors were denervated bilaterally by cutting the cervical sympathetic nerve trunks and the superior laryngeal nerves (Krieger 1964) The rats were paralyzed with Flaxedil (2–4 mg/kg) and ventilated artificially with pure oxygen. The ventilation was kept at a level where the arterial PCO_2 was around 40 mmHg Small amounts of HCO were given i v to prevent acidosis After thoracotomy the right middle lung vein was cannulated *with a thin catheter (PE 10)* and left atrial pressure was recorded continuously The left kidney was exposed retroperitoneally by a flank incision and a bundle of the renal nerves was dissected free over a length of about 1 cm under a dissecting microscope The nerve bundle was freed from fat and connective tissue and it was placed on a pair of thin silver electrodes The nerves and the electrodes were isolated with Neo-Galt (Royce Dental Products Ltd) The rectified mean renal nervous activity (µV) was recorded continuously on a Grass polygraph (Mod 7) together with the left atrial pressure and the arterial blood *pressure* The rats were heparinized (300 IE/kg b w) and placed on a thermostatically controlled heat pad and the body temperature was kept at 37 to 38°C. To test if the baroreceptors were adequately denervated a small amount of norepinephrine enough to raise the blood pressure 20–30 mmHg was given i If this did not affect the renal nervous activity the baroreceptors were said to be denervated Continuous infusion of horse plasma was started at *the rate of 1.8 ml/min*

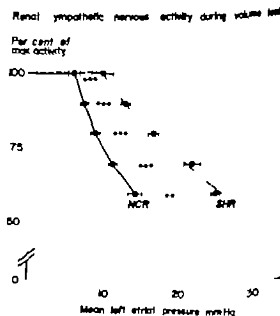


Fig 2 The relationship between mean left atrial pressure and mean rectified renal nervous activity in 6 NCR and 6 SHR during volume load with *horse plasma.* The changes in renal nervous activity are expressed as per cent of maximal control activity (**$P<0.001$)

nerve traffic from left atrial C-fiber endings in SHR similar resetting of the receptors was observed Thorén et al. 1978*b*)

What is then the cause of the resetting of the cardiac receptors? It was recently shown by Noresson et al. (1978) that the mean left atrial pressure was considerably higher in SHR (10 3 mmHg) in comparison with NCR (4 6 mmHg). This elevated pressure in the left atrium and the pulmonary veins should lead to a physical stress on the left atrium and the intrathoracic veins with a compensatory hypertrophic adaptation of the mesenchymal elements of the walls as shown in other parts of the vascular system in SHR (e.g. Folkow et al. 1973). The cause of the resetting of the atrial receptors in SHR might thus be an altered distensibility of the atrial wall.

The resetting of the left atrial receptors (Fig.) will be influenced by a postulated decreased distensibility of the venous system in SHR. Thus, the relationship between changes in plasma volume and left atrial pressure was markedly shifted to the left in SHR, meaning that a certain increase in plasma volume induced a greater rise in left atrial pressure (Fig. 3 A). This decreased distensibility of the venous system in SHR is probably due to stiffer pulmonary veins, secondary to the chronic elevation of left atrial pressure but of course a decreased distensibility of the systemic veins can contribute.

Interestingly enough, an altered distensibility of the venous system in SHR will shift the curve relating changes in plasma volume and renal nervous inhibition to the left (Fig. 3B). Thus, a certain change in plasma volume evoked a greater inhibition of sympathetic outflow in SHR than in NCR. This finding might be explained by a greater change in distensibility of the pulmonary and/or the systemic veins than in the left atrium per se.

The resetting of the atrial receptors in SHR is probably of great functional importance because Hallbäck et al. (1975) showed that the Frank Starling relationship between left atrial pressure and left ventricular stroke volume was shifted to the right in SHR because of the hypertrophy of the left ventricle. The hypertrophied heart in SHR really needs the elevated left atrial pressure to keep up the stroke volume and such a high left atrial pressure in the absence of resetting of the atrial receptors is likely to trigger a marked peripheral vasodilatation and venodilatation. SHR would then be unable to keep up the required high filling pressure of the left ventricle.

The cardiac C-fibre endings are also likely to be of importance for the reflex neural control of renal function (Thorén 1979) via their effects on sympathetic outflow to the kidney. Changes in sympathetic outflow will then also influence renal sodium handling (DiBona 1977). The observed greater reduction in renal nerve traffic to a certain change in plasma volume in SHR might then be one contributing factor to the exaggerated natriuresis observed upon volume load in hypertensive animal and patients.

In summary there is a clear resetting of the cardiac receptors in the SHR with established hypertension. The nature of this resetting is probably the same as for the arterial baroreceptors i.e. an altered distensibility of the left atrial wall where the receptors of functional importance are localized. This resetting is likely to be of functional importance for SHR allowing an adequate filling pressure for the left ventricle and also for the neural control of renal function triggered from cardiopulmonary receptors.

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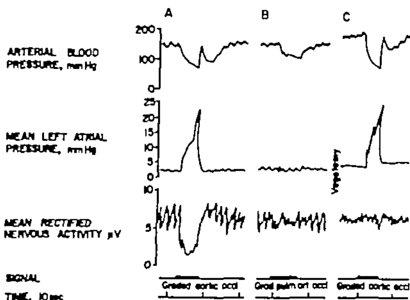


Fig 4 Recordings of arterial blood pressure mean left atrial pressure and mean rectified renal nervous activity during graded aortic occlusion (*A*) pulmonary artery occlusion (*B*) and graded aortic occlusion after bilateral cervical vagotomy (*C*) in a normotensive rat

3 Location of receptors Fig 4 is a recording from an expt where snares have been placed around the ascending aorta and the pulmonary arterial trunk of a normotensive control rat. This made it possible to selectively change the pressure in either the left or the right side of the heart. In this expt (Fig 4) the nervous activity was registered with a low time constant (0.3 s) in order to record rapid changes of the nervous activity Aortic occlusion (Fig 4 A) decreased the renal nervous activity drastically and rapidly Pulmonary artery occlusion (Fig. 4 B) had no effect on the nervous activity After vagotomy (Fig 4 C) occlusion of the aorta did not inhibit the nervous activity Thus the responsible receptors are located in the left side of the heart

The inhibition of renal nerve traffic upon volume load was mediated via the vagal nerves because upon bilateral vagotomy the renal nerve traffic returned to preinfusion values as tested in 5 expts

DISCUSSION

These experiments indicate that cardiopulmonary receptors in rats can effectively inhibit the renal sympathetic nervous activity upon activation in the same way as earlier reported in e g rabbits (Clement 1972) cats (Thorén et al 1976) and dogs (Mancia et al 1973) The vasomotor inhibition is mediated via the vagal nerves because bilater vagotomy abolished the inhibition from the intr thoracic low pressure receptors From the plas infusion expts it is difficult to conclude wheth the receptors are located in the right or the le side of the heart as volume load will increase th pressure in all cardiac chambres However th fact that aortic occlusion but not plumonary arter occlusion inhibited renal nerve traffic indicated th the receptors were located mainly in the left side the heart.

Recently Thorén et al (1978*a*) recorded activi from left atrial receptors in the rat The left atri had a substantial population of endings respondi to atrial distension Peculiar enough all the endin had afferent fibres within the C fiber group. N atrial medullated fibres or ventricular endings wer found It is thus likely that these left atrial C-fibr endings were responsible for the tonic vasomot inhibition from the left side of the heart describ in the present study

This study also showed that there is a clo resetting of the cardiac receptors in SHR (Fig because the threshold in left atrial pressure for ren nervous inhibition was twice as high in SHR com pared with NCR (Fig .) Furthermore when rela ing the left atrial pressure and the renal nervous in hibition for NCR and SHR this curve was clear shifted to the right in SHR When recording th

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The effect of glucagon dibutyrylic cyclic AMP and theophylline on bile production in the cat

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LARSEN J A KRARUP N. & THOMSEN O Ø The effect of glucagon dibutyrylic cyclic AMP and theophylline on bile production in the cat. Acta Physiol Scand 1979 106, 23–27 Received 14 Sept. 1978. ISSN 0001-6772 Institute of Physiology University of Aarhus Denmark.

The effect of glucagon, dibutyrylic cyclic AMP and theophylline on bile production and liver metabolism was studied in fasting, chloralose anesthetized cats. After 45 min infusion of glucagon (0 1 μg/kg/min) total bile flow started t increase and finally reached a level 32% above control bile flow The rise in flow was accompanied by parallel increase in the biliary clearance of erythritol and the rate of biliary excretion of inorganic ions whereas the bile acid excretion remained constant. Glucagon therefore appears to stimulate selectively the bile acid-independent canalicular production of bile In contrast to the delayed action on bile production, glucagon caused an immediate change in liver metabolism as judged from the elimination rate of ethanol and the rise in plasma glucose concentration Dibutyrylic cyclic AMP or theophylline also caused similar immediate changes in liver metabolism but neither substance influenced bile production or the biliary excretion of electrolytes or bile acids. It thus appears that glucagon choleresis in the cat is either independent of cAMP release or that an increase in intracellular cAMP is not in itself sufficient to influence bile secretion The results also seem to exclude that an increase in insulin production induced by hyperglycemia, or hemodynamic changes in the liver can explain glucagon choleresis.

Key ords. Bile production, glucagon, cyclic AMP theophylline

Glucagon stimulates bile flow in man (Dyck & Janowitz 1971 Levine & Hall 1976) and dogs (see Barnhart & Combes 1975) In rats Balabaud et al. (1976) found a choleretic effect of glucagon whereas others did not (Shaw & Heath 1973 Baker & Kaplan 1976). The information about the mechanism of the choleretic action of glucagon is controversial Some of the experiments indicate that glucagon stimulates the bile acid-independent fraction of canalicular bile production, while others suggest a stimulation of bile acid secretion or a secretin-like effect on bile ductuli (see Forker 1977).

In experiments on cats (Krarup & Larsen 1974) glucagon induced marked changes in liver metabolism and splanchnic hemodynamics which could be mimicked by dibutyrylic cyclic AMP (DBcAMP) Krarup et al. 1975) but neither substance had significant effect on total bile flow However a possible influence on canalicular or ductal bile production was not examined.

The purpose of the present experiments was therefore to re-examine the effect of glucagon and DBcAMP on liver function in cats with special reference to an effect on the production of bile This was accomplished by extending the period of observation and by supplementing the previous experimental procedures with the determination of the biliary clearance of ^{14}C-erythritol which is supposed to be a measure of the canalicular production of bile The effect of theophylline on bile production was also examined.

METHODS

12 cats (weight 2.9±0 2 kg) were used for the experiments. The cats were fasted overnight but had free access to water They were anesthetized with chloralose (50 mg/kg)

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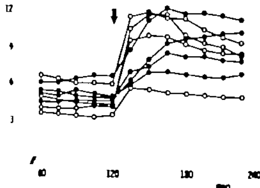


Fig. 2 Arterial plasma glucose concentrations during experiments in which infusion of glucagon, 0.1 μg/kg/min (O) or DBcAMP 100 nmol/kg/min (●) was started as indicated by the arrow

...limination curves (Fig. 3) was immediately increased by glucagon (from 33±4 to 41±2 μmol/...g/min)

During the infusion of DBcAMP no change in bile flow biliary excretion of electrolytes, bile acids or ¹⁴C-erythritol clearance could be detected. In contrast, DBcAMP increased the arterial concentration of glucose (Fig. 2) and elimination rate of ethanol (Fig. 3) (from 35±3 to 42±2 μmol/kg/min. These changes were of the same magnitude as those produced by glucagon.

Theophylline (14 μmol/kg + 1 μmol/kg/min) caused a slight, but not significant rise in bile flow from 11.0±1 7 to 12.–±1 0 μl/kg/min) and erythritol clearance (from 13.5±0 9 to 14 9±0 7 μl/kg/min). Likewise no significant change in the biliary excretion of electrolytes and bile acids was observed. In contrast to glucagon and DBcAMP theophylline did not affect the arterial concentration of glucose or the elimination rate of ethanol. When the dosage of theophylline given was raised 5 to 10 times an increase in arterial glucose concentration and the elimination rate of ethanol was seen. However no further effect on bile production and the biliary excretion of electrolytes or bile acids was observed. On the contrary the slight increase in these parameters was followed by a significant fall possibly secondary to effects on the cardiovascular system manifested as a gradual decrease ...erial mean blood pressure

DISCUSSION

The present experiments have demonstrated that, compared with studies in man and dogs glucagon choleresis in the cat has a more gradual onset and the rise in bile flow does not become significant until about 45 min after the start of the glucagon infusion. This explains why the choleretic effect of glucagon was not noticed in previous experiments (Krarup & Larsen 1974) where the observation period was much shorter

The results indicate that glucagon selectively stimulates the bile acid-independent fraction of canalicular bile production in the cat in accordance with the findings of Khedis et al. (1974) and Barnhart & Combes (1975) in dogs and of Balabaud et al. (1976) in rats This effect of glucagon is quite different from the choleretic effect of secretin which although structurally similar to glucagon, mainly influences the net ductular fluid transport with a marked increase in the biliary excretion of bicarbonate This well known effect of secretin has also been demonstrated in cats (Larsen & Krarup 1978) and the choleretic effect of these two hormones is therefore clearly distinguishable in this species In this context it is of interest to note that, like other secretagogues acting on exocrine glands, glucagon has been found to cause hyperpolarization of the liver cell membrane in the superperfused mouse liver whereas secretin did not affect the cell membrane potential (Petersen 1974)

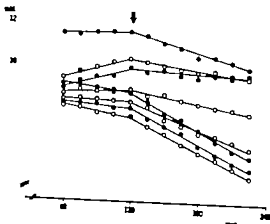


Fig. 3 Arterial elimination curves of ethanol from experiments in which infusion of glucagon 0.1 μg/kg/min (O) or DBcAMP 100 nmol/kg/min (●) was started as indicated by the arrow Lines best fitting the points were drawn by hand

after an initial dose of Nembutal (30 mg) given i.p The abdomen was opened through a midline incision the pylorus ligated and the choledochus cannulated after ligation of the cystic duct. Catheters for infusion were placed in the femoral veins and for blood sampling and blood pressure measurement in a femoral artery Mean arterial blood pressure was recorded using a condensor manometer (Elema Schönander) Body temperature was maintained at 38 5°C by external heating

Ethanol was given as a priming dose (6.5 mmol/kg) followed by a continuous infusion of 36 μmol/kg/min A priming dose of unlabelled erythritol (0 25 mmol) and ¹⁴C-erythritol (3 μC) was also given and this was followed by continuous infusions of ? μmol/min and 33 nC/min respectively Furthermore taurocholate 0 2 μmol/kg/min was given i v

After a recovery and equilibration period of 60 min, arterial blood samples were drawn and bile was collected at 15 min intervals After a 60 min control period glucagon (0 1 μg/kg/min) DBcAMP (100 nmol/kg/min) or theophylline (14 μmol/kg + 1 μmol/kg/min) was infused during the next 1?0 min Four cats were given glucagon 4 DBcAMP and 4 theophylline

Blood samples were analyzed for glucose and ethanol and the biliary concentrations of bile acids sodium potassium chloride and bicarbonate ions were determined ¹⁴C-activity in bile and plasma were also measured in all specimens. The analytical methods have previously been described (Krarup et al 1976)

Calculations

The elimination rate of ethanol was determined as the infusion rate of ethanol corrected for the amount retained in or disappearing from the solvent space per minute the solvent space being set as 68% of the body weight (Krarup & Larsen 197?) The biliary clearance of ¹⁴C erythritol was determined from bile flow and bile to plasma ratio of ¹⁴C-activity The validity of this parameter as a measure of the canalicular bile production has previously been discussed (Krarup et al 1976) Biliary ex creations of bile acids and inorganic ions were calculated from bile flow and the biliary concentrations of the ions.

Statistical proced e

The effects of glucagon DBcAMP or theophylline were tested by the Student s t-test for paired comparisons In the results mean values and S E are given

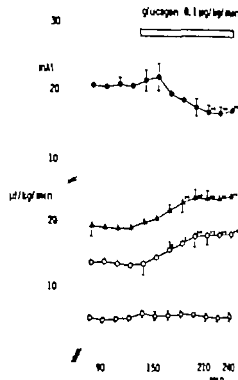


Fig 1 Effect of glucagon (0 1 μg/kg/min) on total bile flow (O) canalicular bile production as measured by the clearance of ¹⁴C-erythritol (Δ) ductular reabsorption of fluid (□) and the biliary concentration of bile acids (●). The points with bars represent mean values and S E. from 4 expts P<0 05 <0 025

RESULTS

It appears from Fig 1 that after 45 min infusion of glucagon bile flow increased significantly and finally reached a new level about 32% above control flow (from 13 6±0 7 to 17 9±0 7 μl/kg/min) It further appears that this increase is fully ac counted for by an increase in the canalicular bile production (from 18 9±0 7 to 23 6±0 7 μl/kg/min) as estimated from the biliary ¹⁴C-erythritol clear ance On the same basis the net fluid transport in the bile ductules was not affected It is also seen from Fig 1 that there was a gradual decline in the biliary concentration of bile acids corresponding to the increase in bile flow Thus the biliary excretion rate of bile acids was not significantly affected by glucagon (0 ?8±0 04 μmol/kg/min in the control period maximum 0.31±0 06 μmol/kg/min during glucagon infusion P>0 05) Slight but significant increases in the biliary concentrations of the inorganic anions were observed (chloride from 104±4 to 106±3 mM P<0 05 bicarbonate from 26±2 to ?8±1 mM P<0 05) whereas no changes in the concentrations of sodium (145±2 mM before and 145±3 mM during glucagon) and potassium (3 6±0 2 mM before and 3 8±0 3 mM during glucagon) occurred This means that the excretion rates of the inorganic ions increased almost in parallel with bile flow

Glucagon caused an immediate increase in arterial glucose concentration which after about 45 min was followed by a slow decline (Fig 2) Ethanol elimination rate as judged from the

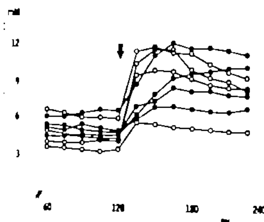


Fig 2 Arterial plasma glucose concentrations during experiments in which infusion of glucagon, 0 1 μg/kg/min (O) or DBcAMP 100 nmol/kg/min (●) was started as indicated by the arrow

elimination curves (Fig. 3) was immediately increased by glucagon (from 33±4 to 41±2 μmol/kg/min).

During the infusion of DBcAMP no change in bile flow biliary excretion of electrolytes, bile acids or ^{14}C-erythritol clearance could be detected. In contrast, DBcAMP increased the arterial concentration of glucose (Fig. 2) and elimination rate of ethanol (Fig 3) (from 35±3 to 42± μmol/kg/min. These changes were of the same magnitude as those produced by glucagon.

Theophylline (14 μmol/kg + 1 μmol/kg/min) caused a slight, but not significant, rise in bile flow (from 11 0±1 7 to 12 2±1 0 μl/kg/min) and erythritol clearance (from 13.5±0 9 to 14 9±0.7 μl/kg/min) Likewise no significant change in the biliary excretion of electrolytes and bile acids was observed. In contrast to glucagon and DBcAMP theophylline did not affect the arterial concentration of glucose or the elimination rate of ethanol. When the dosage of theophylline given was raised 5 to 10 times an increase in arterial glucose concentration and the elimination rate of ethanol was seen. However no further effect on bile production and the biliary excretion of electrolytes or bile acids was observed. On the contrary the slight increase in these parameters was followed by a significant fall, possibly secondary to effects on the cardiovascular system manifested as a gradual decrease in arterial mean blood pressure.

DISCUSSION

The present experiments have demonstrated that, compared with studies in man and dogs, glucagon choleresis in the cat has a more gradual onset and the rise in bile flow does not become significant until about 45 min after the start of the glucagon infusion This explains why the choleretic effect of glucagon was not noticed in previous experiments (Krarup & Larsen 1974) where the observation period was much shorter

The results indicate that glucagon selectively stimulates the bile acid-independent fraction of canalicular bile production in the cat in accordance with the findings of Khedis et al (1974) and Barnhart & Combes (1975) in dogs and of Balabaud et al. (1976) in rats This effect of glucagon is quite different from the choleretic effect of secretin which, although structurally similar to glucagon, mainly influences the net ductular fluid transport with a marked increase in the biliary excretion of bicarbonate This well known effect of secretin has also been demonstrated in cats (Larsen & Krarup 1978) and the choleretic effect of these two hormones is therefore clearly distinguishable in this species. In this context it is of interest to note that, like other secretagogues acting on exocrine glands glucagon has been found to cause hyperpolarization of the liver cell membrane in the superfused mouse liver whereas secretin did not affect the cell membrane potential (Petersen 1974).

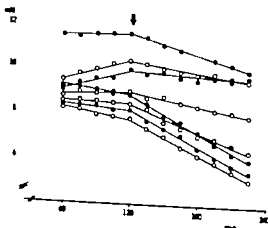


Fig 3 Arterial elimination curves of ethanol from experiments in which infusion of glucagon 0.1 μg/kg/min (O) or DBcAMP 100 nmol/kg/min (●) was started as indicated by the arrow Lines best fitting the points were drawn by hand.

This indicates that glucagon acts on liver cells via an interaction with specific liver membrane receptors Furthermore secretin does not bind to glucagon receptors in rat liver membranes (Rodbell et al 1971) and glucagon does not bind to secretin receptors of the pancreatic acinar cells from guinea pigs (Christophe et al 1976)

It is well known that glucagon increases the hepatocytic cAMP content by stimulating adenyl cyclase and a similar rise can be obtained by administration of DBcAMP or theophylline (Robinson et al 1971) It might therefore be assumed that glucagon affects the secretion of bile via intra cellular liberation of cAMP This is supported by the experiments of Petersen (1974) in which it was demonstrated that the hyperpolarization of the liver cell membrane could also be induced by DBcAMP and furthermore the dose response relationship of the glucagon effect on membrane potential and on adenyl cyclase activity was similar It has also been demonstrated that the choleretic effect of glucagon in man can be reproduced by infusion of DBcAMP whereas theophylline was without effect on bile production (Levine & Hall 1976) In dogs both DBcAMP and theophylline increased bile flow (Morris 1972, Erlinger & Dumont 1973 Barnhart & Combes 1975)

This proposed mechanism of glucagon choleresis appears less obvious in the present experiments on cats The glucagon effect on bile flow was not fully developed until at least 60 min after the infusion of glucagon was begun This might be thought to be due to a delay in the liberation of cAMP but judging from the immediate effect on arterial glucose con centration and on the rise in elimination rate of ethanol no such delay was present If it is accepted that these metabolic effects are mediated by cAMP The fact that infusion of DBcAMP has similar metabolic effects indicate that they are Further more DBcAMP did not affect bile secretion despite its marked metabolic effects on the liver and nor did theophylline influence bile production The results therefore lead to the unexpected conclusion that glucagon choleresis in the cat is either independent of cAMP release or is mediated by cAMP in com bination with one or more unknown factors

It is known that in the cat (Larsen & Christensen 1978) as well as in the dog (Snow & Jones 1978) insulin affects mainly if not exclusively the bile acid independent fraction of canalicular bile production It might therefore be argued that the effect of glucagon on bile production is secondary to liberation of insulin caused either by hyperglycemia or by a direct stimulatory effect of glucagon on insulin production The present experiments indicate that a liberation of insulin induced by hyperglycemia is not very likely since infusion of DBcAMP produced the same rise in plasma glucose as seen with glucagon without affecting bile production. However a direct stimulatory action of glucagon on insulin production cannot be excluded.

It should finally be noticed that glucagon in doses similar to those used in the present experiments caused characteristic changes in splanchnic hepatic hemodynamics which could be mimicked by DBcAMP (Krarup & Larsen 1974 Krarup et al 1975) It therefore seems unlikely that hemodynamic changes should be of importance for the choleretic effect of glucagon

We gratefully acknowledge the skilled technical assistance of Miss Karin Donby

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The effect of noise on cochlear blood flow in the conscious rabbit

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HULTCRANTZ, E. The effect of noise on cochlear blood flow in the conscious rabbit. Acta Physiol Scand 1979 106: 29–37 Received 24 Oct. 1978. ISSN 0001-6772. Department of Otolaryngology University Hospital, Uppsala and the Institute of Physiology and Medical Biophysics, Biomedical Centre, University of Uppsala, Sweden.

The cochlear blood flow was measured with the microsphere method in unanesthetized rabbits, prior to and during noise-exposure. Different sound pressure levels were used. 100 dB, 115 dB and 120 dB Some of the animals had the cervical sympathetic trunk cut unilaterally The effects of the delivered noise on the organ of Corti were studied in other rabbits with scanning electron microscopy In spite of ultrastructural changes indicating damage of sensory cells after 115 dB and 120 dB exposure no changes of the total cochlear blood flow could be observed. After unilateral section of the cervical sympathetic trunk the cochlear blood flow was less on the intact side (25%) already prior to noise exposure and the difference was not significantly affected by noise. Different sizes of microspheres were used. 8.2, 8.4 8.7 9.2 μm The 8.2 and 8.4 μm-spheres seemed well fitted for measurements of cochlear blood flow but passed the cerebral capillaries to a high extent. Control experiments using both 8.2/8.4 μm-spheres and 15 μm-spheres were performed and the degree of passage in different tissues was estimated.

Key words. Cochlear cerebral blood flow noise, microspheres, sympathetics

The manner by which noise damages the inner ear is still not clearly elucidated. Some theories indicate that noise may cause a deteriorated blood flow to the cochlea (Yoshioka 1957 Perlman & Kimura 196., Kellerhals 1972, Maass et al. 1976, Maass 1977) and in that way diminish the oxygen supply to the organ of Corti, causing damage to the hair cells. Angelborg, Hultcrantz & Beausang-Linder (in press) could not support this theory when using the microsphere method for quantitative measurements of the total cochlear blood flow in anesthetized cats. Since anesthesia may diminish the degree of damage to hair cells during acoustic trauma (Rubenstein 1976) and influence the distribution of the cardiac output in many ways (Landau et al. 1955 Goldman & Saperstein 1973 Bell et al 1976, Marcus et al 1976), it was considered important to study ( ) whether noise of different sound pressure levels (SPL) could influence the cochlear blood flow in unanesthetized animals, (*b*) whether it was possible to correlate the degree of hair cell damage to effects on the cochlear blood flow and (*c*) if and how the sympathetic nervous system reacted to noise.

MATERIAL AND METHODS

35 young, healthy albino rabbits, weighing between 1.4–2.4 kg were used. On day 1 twenty-nine of the animals were anesthetized with pentobarbital sodium before surgery Polyethylene catheters were inserted into the femoral arteries and catheter was placed into the left ventricle of the heart via the left brachial artery The site was controlled by recording the blood pressure, studying the pulse wave, which increases when passing the aortic valves The catheters were pushed through the subcutaneous tissues and out through the skin on the animal' back, where they were fixed in place On six of the animals the cervical sympathetic trunk was cut proximal to the superior ganglion. The vagal nerve was not touched A sham operation was performed on the opposite side

On day 2 the animals were placed with the head into sound insulated box connected to loudspeaker Holes in the box allowed oxygen or air supply and prevented CO_2-

Table 1 *The different experimental and control groups with respect to noise exposure sympathectom si e of used microspheres*

Groups	Noise exposure		Sympathectomy	No of animals	Microspheres, mean diameter in μm	
	1st measurement	2nd measurement			1st injection	2nd injection
Experimental						
I	–	100 dB	–	3	9.2	8.8
				4	8 4	8.2
II	–	115 dB	–	3	9..	8 8
				1	8 4	8.2
III	–	1?0 dB	–	3	8.4	8.2
IV	–	100 dB	Unilateral	6	8 4 and 14 5	8.2
Controls						
V	100 dB	–	–	1	8.4	8.2
				1	8.2	8.4
VI	–	–	–	1	8.4	8.2
				1	8.2	8 4
VII	–	100 dB	–	5	14 4	8 4 and 14.5

retention without any significant reduction of sound pressure level during noise exposure One of the femoral catheters was connected to a pressure transducer and the blood pressure was recorded From the other femoral catheter a blood sample for acid-base control was taken and analyzed in an acid-base laboratory ABL 1 (Radiometer Copenhagen) The first blood flow measurement was performed by injecting radioactively labelled microspheres intracardially during approximately 10 s and simultaneously sampling blood by free flow from the femoral artery The blood sampling continued during exactly 1 min (Alm & Bill 1972 Hultcrantz et al 1977) After the first measurement, the animals were exposed to noise for 6 min and the second measurement was performed during the last minute using differently labelled microspheres The acid-base balance was controlled a second time just before the last blood flow measurement. Immediately after the end of the blood sampling, the animals were anesthetized with pentobarbital sodium i and killed by intracardial injection of saturated KCl. The cochleas were removed and fixed in sodium phosphate buffered 2.5% glutaraldehyde before microdissection (Angelborg et al 1977 Hultcrantz & Angelborg 1978)

The brain was dissected in different parts including medulla oblongata, inferior colliculi, auditory cortex Specimens from kidneys heart and masseter muscles were taken After counting the radioactivity in the different tissues and in the reference blood samples the blood flow could be calculated using the formula

$$\frac{f}{n} = \frac{f_{ref}}{n_{ref}}$$

f = blood flow in the cochlea mg min f_{ref} = the reference blood flow mg min mg n = radioactivity of the cochlea, n_{ref} = radioactivity of the reference blood sample.

The rabbits were divided into groups (see Table 1) with respect to the SPL they were exposed to: 7 animals were exposed to 100 dB 4 to 115 dB and 3 to 120 dB and sympathectomized animals, which were all exposed to dB Nine animals were used to check the method ... them were measured twice with a 5 min interval without being exposed to noise and two were measured first during 100 dB SPL and a second time 5 min after the end of the exposure A further 5 animals were measured with microspheres that were twice the size of the others, below The same standardized tape with equalized ... white noise was used in all the experiments (Angelborg et al in press) Two cochleas were excluded due to damage during dissection.

Microspheres

The microspheres used were carbonized latex particles (3M Co Minneapolis Minnesota) labelled with either ^{141}Ce or ^{85}Sr Two batches of each kind of spheres were used during the series the mean diameters and variances differing between the batches. Spheres with mean diameter ±S D 9.2±0 6 μm and 8.8±0.9 μm were used in ... experiments and spheres with mean diameter 8.2±0 8 ... and 8.4±0.5 μm in the others (Table 1) The number of spheres injected each time was appr 17×10⁶ 21±10⁶ dispersed in 1.5 ml 0 9% saline The spheres were warmed to body temperature and aggregates were dispersed by ultrasound before injection

As the smaller spheres used (8.2±0 8 μm and 8 4±

...m) appeared to pass through the capillary net in the brain a considerable extent, the five rabbits in group VII (able 1) were used to check these results: the animals prepared and noise-exposed (100 dB) as the others at in the first blood flow measurement microspheres of 1 0 μm diameter were used (^{141}Ce) and in the second mixture of 8 4±0.5 μm-spheres (^{85}Sr) and 14 5±0 8 μm spheres (^{46}Sc). The number of spheres in the first injection as appr 10^{6} and in the second appr 5×10^{6} of the smaller ones and 1 10^{6} of the larger For the same reason ... animals in group IV were injected in the first measurement with mixture of small spheres (8 4±0.5 μm) in number of 21 10^{6} and spheres sized 14 5±0.8 μm number of 1 10^{6}

...anning Electron Microscopy

... rabbits were used to study the effect of the delivered ...se on the organ of Corti, 2 at each SPL (100 115 and ...0 dB) One of each pair was put to death immediately ...ter 6 min exposure and the other after 2 weeks of survival. They were put to death by an anesthetic dose of ...entobarbital sodium followed by saturated KCl i... ...ithin 5 min after death the cochleas were fixed in sodium phosphate buffered 2 5% glutaraldehyde. The cochleas ...ere microdissected after being post fixed in veronal buffered 1 5% OsO$_4$. The specimens were dehydrated and ... according to the critical point method (Andersson ...951) and coated with gold with sputtering technique A ...ulips Scanning Electron Microscope 501 was used for study of the surface structure of the organ of Corti

RESULTS

All the rabbits survived the surgery Some of them had a metabolic acidosis, the mean value ±S D of the pH values was 7.38±0 05 None had a carbon dioxide retention, the mean ±S D of the PCO$_2$ was ... 95±0.78 kPa. The acidotic animals got an O$_2$-supply resulting in a PO$_2$ in arterial blood up to 45 kPa. The others had a PO$_2$ in arterial blood around 12 kPa. The mean arterial blood pressure was between ...0 and 14 kPa. When the head was placed into the ...ox the animals exhibited a brief rise of blood pressure and tachycardia. This rise only lasted a few seconds and the blood pressure returned to the initial level before the blood flow measurement. When the noise exposure started all animals again exhibited rise of blood pressure but they generally ...abilizated within half a minute to the initial level ... of the animals exposed to 115 dB reacted with ... fall of blood pressure and bradycardia.

Blood flow measurements

No effect of noise on the total cochlear blood flow could be registered in any of the groups (Fig 1) The mean cochlear blood flow ±S D in 23 cochleas was 2.26±0.67 mg min before noise and 2.1±0 67 mg min^{-1} during noise exposure The change in flow was calculated for each cochlea as: flow during noise minus flow before. The mean difference and S.D was 0.04±0 45 which was not statistically significant. Three noise-exposed cochleas not included above exhibited a condition of sclerotic otitis media. Their mean flow was 0.92 mg min^{-1} range: 0.6–1.2 during the first measurement and 1.55 mg min (0.9–1.96) during noise.

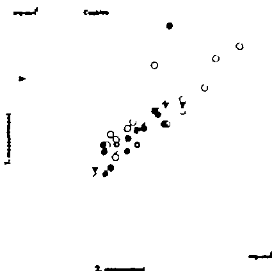


Fig 1 Cochlear blood flow The relation between two measurements. The line is that of identity O=cochleas exposed to 100 dB during the second measurement. ● cochleas exposed to 115 dB during the second measurement. ◐ cochleas exposed to 120 dB during the second measurement ● =cochleas exposed to 100 dB during the first measurement. ▼=cochleas not exposed to noise.

No systematic difference could be seen between the two measurements in the two control animals which were not exposed to noise of in the two which were measured first during noise and the second time after noise (Fig. 1). No significant difference of the mean cochlear blood flow was found between the 10 cochleas measured with 9.2/8.8 μm-spheres and the 13 cochleas measured with 8.2/8 4 μm-spheres.

The cochlear blood flow in the 6 animals with unilateral section of the cervical sympathetic trunk is presented in Fig. 2. There was a significant difference between the sympathectomized side and the intact side of 25±7% ($p<0$ 05) This difference decreased to 16±5% during the second measurement.

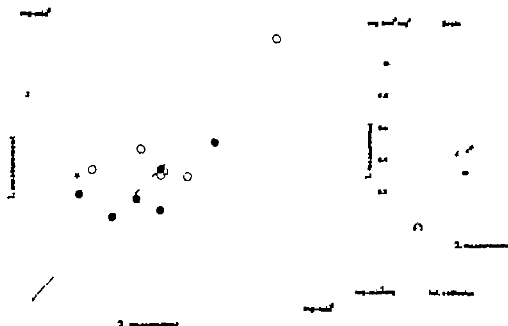


Fig 2 Cochlear blood flow *The effect of sympathectomy* Two measurements the first before and the second during noise exposure (100 dB) The line is that of identity O = cervical sympathectomized side ● = sympathetical intact side ★ indicates one animal with haemorrhagic shock *during the second measurement*

The values for cerebral blood flow obtained with 8 2–9 2 μm-spheres are presented in Fig 3*a* and the blood flow to the inferior colliculi in Fig 3*b* In almost all cases the second measurement gave a higher value than the first regardless whether the animal was noise-exposed or not. When 9 2/8 8 μm spheres were used the mean values were significantly higher than the values obtained with 8.2/8 4 μm-spheres ($P<0\ 001$)

The renal blood flow decreased by 25% during noise exposure In the two cases where the second measurement was performed 6 min after noise-exposure a 16% increase was noticed The two animals measured twice without any noise exposure did not show any significant change of the renal blood flow

The blood flow values measured with 14.5/14 4 μm spheres for the different parts of the brain in 5 of the control animals (group VII Table 1) are presented in Table 2 The mean change of the total cerebral blood flow was −15% which was not statistically significant but the mean change in the inferior colliculi was +25% range −7 to +54% during noise stimulation which was *significant* ($P<0\ 05$)

The mean renal blood flow measured *with 14.5/*

Fig. 3 (*a*) Total cerebral blood flow in relation to n… exposure and sphere-size used. The line is that of identi… (*b*) Blood flow in inferior colliculi. Mean value for th… sides of each animal ▲ = animals exposed to noise dur… the second measurement sphere-size *9.2/8 8 μ…* △ = animals exposed to noise during the second measure*ment, sphere size 8.4/8 2 μm.* O = animals exposed … noise during the first measurement sphere-size 8 4… μm. ● = animals measured twice without noise-exposur… sphere-size 8 4/8 2 μm.

14 4 μm-spheres decreased by 44% range 6–66 … during noise exposure ($P<0\ 05$) and the blood flo… of the myocard increased moderately in four of th… animals but decreased in one In all the animal… there was a decrease of the blood flow to the masse… ter muscles during noise mean decrease 38… range 10–94%

Fig 4 shows the results from the same animal… where simultaneous measurements with 8 4±… μm spheres and 14 5±0 8 μm-spheres were pe… formed The blood flow values obtained by th… smaller spheres are expressed in percent of the va… ues obtained with the larger ones The smalle… *spheres gave significantly lower values than th…*

able 2. M asureme ts of cerebral blood flow prior to and during noise exposure (100 dB)

licrospheres sized 14.4±1.0 μm and 14.5±0 8 μm. Blood flow expressed in mg min⁻¹ mg Air respiration. I = first ieasurement, II = second measurement

	Experiment number					
	1	2	3	4	5	±S.D
erebrum						
Anterior part						
I	0.80	1 14	0 75	1 14	0.58	0.88±0.25
II	0.78	0.74	0.93	0.72	0.67	0 77±0.10
Posterior part						
I	0.44	0.94	0.64	1 08	0.49	0.72±0 28
II	0.56	0 60	0 65	0 62	0 52	0.59±0.05
Auditory cortex						
I	0.81	1 17	1 09	1.58	0.73	1.08±0 34
II	1 12	0.84	1 18	0.81	0.83	0.96±0.18
nferior colliculi						
I	0.85	1.23	1.23	0.82	1.06	1.04±0.20
II	1.31	1 14	1.54	0.90	1.63	1.30±0.30
Pons and medulla obl.						
I	0.53	0.74	0 73	0 82	0.49	0 66±0.14
II	0.74	0.59	0.84	0.76	0.59	0.70±0.11
erebellum						
I	0.55	0.97	0.57	1.06	0 60	0 75±0..4
II	0 67	0.73	0 66	0.75	0.67	0.70±0.04

arger ones in the brain, indicating poor trapping of the smaller ones In the experiment with mixing of 8.4+0.5 μm and 14.5±0.8 μm-spheres in the first measurement (group IV Table 1) the difference in flow values was about 50% in the CNS No figures can be given for the cochleas in this context, since the number of 14.5 μm-spheres used did not permit accurate measurements of the cochlear blood flow

Scanning Electron Microscopy

No abnormal findings could be seen in the organ of Corti in the two animals exposed to 100 dB SPL (Fig. 5)

In the pair of animals exposed to 115 dB SPL no damage could be seen in the rabbit immediately after exposure The one which survived 2 weeks before being killed showed degeneration of the cilias and some cell losses 1 1..5 turns from the cochlear base (Fig. 5*b*) The inner hair cells were intact.

The two animals exposed to 120 dB SPL both showed pronounced signs of damage in the whole of the organ of Corti. The animal killed immediately showed some disarrangement and loosening of the cilias on the outer hair cells and the one which was put to death 15 days after exposure had a most extensive damage at 1–1.25 turns from the base, where both outer and inner hair cells were damaged. However cell losses and degeneration of cilias among the outer hair cells were also seen in the other parts of the cochlea (Fig. 5*c*)

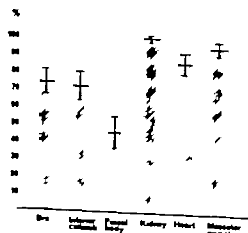


Fig 4 Blood flow simultaneously measured with two sizes of microspheres. 8.4±0.5 μm and 14.5±1.0 μm in diameter The blood flow values for the smaller spheres in percent of the values for the larger Mean and S.D 5



Fig 5 The effects of 6 min noise exposure on the sensory cells of the organ of Corti The animals killed weeks after the exposure From top to bottom 100 dB SPL, 115 dB SPL, 120 dB SPL. Marker 10 μm

DISCUSSION

The cerebral blood flow is greatly influenced by different anesthetics (Bell et al 1976 Marcus et al 1976) and the same might be true for the cochlear blood flow Earlier quantitative studies of the cochlear blood flow have all been made on anesthetized animals (Angelborg et al 1977 Hultcrantz et al 1977 Hultcrantz & Angelborg 1978 Angelborg et al in press and Beausang-Linder & Hultcrantz to be published) and the magnitude of reactions may have been influenced by the anesthesia.

In the present study anesthesia was not used and the cochlear blood flow was 2 3±0 7 mg min

bbits anesthetized with pentobarbital sodium and which one cervical sympathetic chain was cut and the other stimulated at 6 Hz the blood flow on the control side was 3.3±1.3 mg min and 2.5± 2 mg min on the stimulated side (Beausang-Linder & Hultcrantz). The cerebral blood flow in the present study was 1.02±0.39 mg min⁻¹ in contrast to that in the anesthetized animals of 0.39±0.1 min⁻¹ mg. Thus, unlike the cerebral blood flow the cochlear blood flow is not depressed by anesthesia.

Ultrastructural changes indicating damage of the sensory cells were observed in the cochleas exposed to 120 dB SPL and to a lesser degree after 15 dB exposure. Since no effect of noise on the total cochlear blood flow could be seen—neither in the animals with unilateral sympathectomy nor in the intact animals—it seems unlikely that vasoconstriction caused by noise was the cause of the observed damage.

Maximum physiological stimulation frequencies of the sympathetic nervous system are around 6 Hz (Folkow & Neil 1971). In previous studies such stimulation resulted in a 25% reduction of cochlear blood flow both in cats and rabbits (Hultcrantz and Angelborg 1978, Beausang-Linder & Hultcrantz, to be published).

In the present experiments in the animals with unilateral sympathectomy there was a 25% difference in flow between the two cochleas already prior to noise exposure and the difference did not increase during noise. When exposed to noise the rabbits usually showed signs of fear initially with rise in blood pressure. The blood pressure normalized within a few minutes however. The reduction in renal blood flow observed during noise suggests that in spite of the normal blood pressure there had been a redistribution of the cardiac output, probably as a result of altered sympathetic activity. The blood flow in masseter muscle was also reduced indicating increased activity in the cervical sympathetic chain. It is somewhat surprising then that noise did not change the blood flow in the cochlea with intact sympathetic innervation. The results make it seem probable that the sympathetic nervous tone of the cochlear vessels is rather high in conscious rabbits under slight stress but not appreciably changed by the further stress caused by noise.

In a previous study in cat under chloralose or ketamin anesthesia sympathectomy had no appreciable effect on cochlear blood flow and noise affected neither the sympathectomized side nor the control side (Angelborg et al. in press). It seems likely then that both anesthetics tended to abolish the sympathetic tone of the cochlear vessels. Noise thus does not seem to affect cochlear blood flow either in unanesthetized animals or in animals under general anesthesia.

The results indicating no effects of noise on total cochlear blood flow appear contradictory to the findings of Misrahy et al. (1955) and Maass (1977) who reported an endolymphatic hypoxia during noise exposure and interpreted this as caused by a reduced blood flow. However an increase in the metabolic rate in the cells of the organ of Corti during noise exposure without a corresponding increase of the blood flow might also lead to an intracochlear hypoxia.

Another cause of hypoxia in the organ of Corti might be a noise-induced redistribution of blood within the cochlea, as suggested by Hawkins (1971) in spite of an unchanged total cochlear blood flow.

It seems of interest to continue studies of the effects of noise on the regional blood flow within the cochlea. However the microsphere method in its present form is probably inexpedient for such a purpose.

The problem is that a relatively large number of microspheres must be present in each investigated region for statistical reasons (Buckberg et al. 1974). As the cochlear blood flow is a very small part of the cardiac output, this necessitates a very large number of microspheres to be injected. Under such conditions the measured blood flow and the general blood circulation may be affected.

Even if an intracochlear hypoxia really is induced during noise exposure it still must be proven whether this hypoxia is the cause of the observed damage to the sensory cells. The most severe damages were seen among the outer hair cells which are reported to use anaerobic glycolysis as their main metabolic pathway (Thalman et al. 1970). As the exposure time was only 6 min it is unlikely that hypoxia was the only cause of the permanent damage to the hair cells.

There was a marked difference in the cerebral blood flow between the animals measured with 9.–±0.6 μm or 8.8±0.9 μm-spheres as compared to those injected with 8.2±0.8 μm or 8.4±0.5 μm-spheres (Fig. 5*a*, *b*). With the larger spheres the values in the first measurement correspond to ear



Fig 5 The effects of 6 min noise exposure on the sensory cells of the organ of Corti. The animals killed 2 weeks after the exposure From top to bottom. 100 dB SPL, 115 dB SPL, 120 dB SPL Marker 10 μm.

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lier findings (Bill 1978) and the increase noticed in the second measurement might have been caused by noise *especially as the most marked increase is* in the inferior colliculi which are parts of the auditory tracts However the smaller spheres (8 2/8 4 μm) gave values in the first measurement which were about half of the expected values for nonanesthetized animals and even the second measurement gave lower values than expected The unexpectedly low values and the difference between the first and second measurements indicated that the 8 2/8 4 μm-spheres were too small to be used for accurate measurements of cerebral blood flow The results with a mixture of small and large spheres suggested that about 25% of the small spheres were not trapped at all in the brain and that another 25% at least loosen during the first 6 min This loss of spheres seemed to be about the same in all the animals regardless whether they were exposed to noise or not.

The critical lower size limit of microspheres useful in measurements of the cerebral blood flow in rabbits seems to be somewhere between 8 4 μm and 8 7 μm For measurements of the cochlear blood flow also the 8 2±0 8 μm-spheres seemed to give reliable results

The effect of noise on the cerebral blood flow was variable in the five animals measured with 14 4/14 5 μm spheres (Table 2) Within the group the noise seems to have equalized the cerebral blood flow the variance is much larger before than during noise stimulation The increase seen in most of the inferior colliculi is in accordance with findings which report regional increase of blood flow in active parts of the brain (Solokoff 1961 Ingvar & Philipson 1977) As the inferior colliculi are parts of the auditory tracts it could be expected that the blood flow should increase during noise stimulation However more studies must be devoted to these problems

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Metabolic responses in feline "red" and "white" skeletal muscle to shock and ischemia

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JENNISCHE, E., AMUNDSON B & HALJAMÄE H Metabolic responses in feline "red" and "white" skeletal muscle to shock and ischemia. Acta Physiol Scand 1979 106. 39-45 Received 30 Oct. 1978 ISSN 0001-6772. Departments of Histology Anatomy and Anaesthesiology I University of Göteborg, Sweden.

In order to investigate possible differences in the reaction to hypoxic conditions between "red" and "white" skeletal muscle cats were subjected to a 2 h period of either hemorrhagic shock or hind limb tourniquet ischemia, and the hypoxia induced changes were studied in the soleus and lateral gastrocnemius muscles. Muscle biopsies were analysed for ATP CP glucose G 6-P and lactate Using microelectrodes, the resting membrane potential was repeatedly measured Both experimental models resulted in increased tissue lactate level and successive decrease in the membrane potential of both muscles studied No reduction of the high energy phosphagen content (ATP CP) occurred in any of the muscles during shock The tourniquet ischemia resulted in a 40% reduction of the ATP+CP content in the soleus muscle, whereas in the gastrocnemius muscle no significant reduction occurred. A significant correlation was found between the tissue lactate content and the membrane potential under both conditions and in both muscles studied It is concluded that "red" muscles are more susceptible to metabolic derangement than "white" muscles during total ischemia, whereas during hypovolemia "red" muscles appear to be protected from early hypoxic damage probably due to a redistribution of skeletal muscle blood flow

Key words: "red" muscle, "white" muscle, hemorrhagic shock, tourniquet ischemia, resting membrane potential tissue metabolites

here are major metabolic as well as microvascular differences between "red" and "white" skeletal muscle. "Red" muscles have a high activity of oxidative enzymes and rely mainly on aerobic metabolism, whereas "white" muscles have a high activity of glycolytic enzymes and a metabolism based partly on anaerobic pathways (e.g. Dubowitz & Pearse 1960 Ogata 1960 Beatty et al. 1963 Lorimand 1965 Bocek et al 1966, Pande & Blanchaer 1971) "Red" muscles have a denser capillary network and a higher resting blood-flow than white muscles (Romanul 1965 Reis et al. 1967 Folkow & Hudlicka 1968, Reis & Wooten 1970 Hilton et al. 1970). There are also differences in the blood flow characteristics (Gray 1971) and in the vascular reactivity to α-adrenergic stimulation the two types of muscle (Folkow & Hudlicka 1968 Hilton et al. 1970, Gray 1971)

ese basic metabolic and microcirculatory differences between "red" and "white" skeletal muscle indicate that differences also in the reaction to hypoxia may be expected In the present study therefore the metabolic response of a "white" muscle the lateral gastrocnemius, was compared to that of a "red" muscle the soleus during hypovolemic shock and during tourniquet ischemia.

MATERIAL AND METHODS

Female cats, weighing 2.0–2.3 kg, were used Anesthesia was induced with diethylether and maintained with chloralose 50 mg/kg b w A tracheostomy was performed to obtain an unobstructed airway but respiration was not assisted One carotid artery was cannulated for continuous blood pressure registration. The animals were placed on a heating pad to keep a core temperature of 37°C. A stabilization period of 1 h was allowed before the start of the experiment. The metabolic response of the soleus and gastrocnemius muscle was studied under the following conditions during 2 h experimental period.

Shock ($n=6$) Hemorrhagic shock was induced by a single withdrawal during 5 min, of 45% of the calculated blood volume (6% of b w)

Tourniquet ischemia ($n=5$) One of the hind limbs was elevated and wrapped with an Esmarch bandage to expel the blood content and a rubber tourniquet was applied around the thigh Occlusion was maintained for 2 h.

Controls The non-ischemic contralateral hind limb of the tourniquet cats served as a control for metabolite analyses and for membrane potential measurements It has been shown previously that no changes occur in the resting membrane potential or in the metabolite levels in the non-ischemic hind limb during tourniquet ischemia (Enger et al 1978)

The following variables were studied

Metabolite levels Small biopsies (20–30 mg w w) were taken from the lateral gastrocnemius and the soleus muscles The biopsies were taken from one of the limbs of animals subjected to shock and from the ischemic as well as the non-ischemic control limb of animals subjected to tourniquet ischemia. The biopsies were frozen in liquid nitrogen homogenized in ice-cold perchloric acid and neutralized with $KHCO_3$ After centrifugation the supernatants were used for fluorometric analyses of ATP phosphocreatine-CP glucose G 6-P and lactate as previously described by Haljamäe & Enger (1975) The pellets were analysed for protein according to Lowry et al. (1951) Metabolite levels are expressed as μmol/g protein. The protein content of the soleus was 14.5±0.3% (mean±S E.) of w w and that of the gastrocnemius 15 4±0.2% of w w

Resting membrane potential The membrane potential was registered repeatedly during the 2 h experimental period in the soleus and in the gastrocnemius muscle using the method described by Campion et al (1969) and modified as previously described by Haljamäe et al (1977) Glass microelectrodes, having a tip diameter of 1–2 μm and a resistance of 5–15 megaohms, were used A small Ag–AgCl electrode placed subcutaneously served as a reference electrode The circuit was completed by a electrometer amplifier and registrations were made on potentiometer recorder (LKB 6530) 8–15 cells were impaled at each occasion Registrations from superficial cells were omitted since they could represent injured muscle cells (Cunningham et al 1971*a*)

Statistical analysis Wilcoxons two-sample rank test and regression analysis were used for the statistical calculations

RESULTS

In Table 1 the metabolite content and membrane potential values of the soleus and the gastrocnemius muscles are shown during initial control conditions after 2 h of hemorrhagic shock and after 2 h of tourniquet ischemia. Control values obtained from the nonischemic control hind limb of the tourniquet exposed animals after 2 h are also included in the Table

Control

The ATP as well as the phosphocreatine-CP content was about 35% lower in the soleus than in the gastrocnemius muscle during control conditions The initial G 6-P content was significantly higher in the gastrocnemius 5 2±0 9 (mean±S E), than in the soleus 2 2±0.5 The lactate content was also higher in the gastrocnemius than in the soleus 18 7±1 6 and 8 0±1 2 respectively whereas the glucose content was essentially the same in both muscles The mean initial membrane potential of the gastrocnemius muscle was −90 0±1.5 mV

Table I *Metabolite content and resting membrane potential of the soleus (Sol) and lateral gastrocnemius (GL) muscle*

	ATP	CP	Glucose	G 6-P	Lactate	Membrane potential
Initial control						
GL $n=6$	52.2±3 0*	187.3± 9 6	15 1±1 3	5 2±0 9*	18 7± 1 6	−90 0±1 5
Sol $n=6$	34.5±1 9	124 0± 9.3	13 4±1.3	2.2±0 5	8 0± 1.2	−87.2±1 1
Shock 2 h						
GL $n=6$	66 9±3 9*	151 0± 8.2	43.5±4 0	26.8±3 7	82 7±11.3**	−81.2±1 4
Sol $n=6$	43.2±3 9	102.1± 7 1	35 9±3 3	14 7±1 9	40.2± 5 7	−75.8±1 4
Ischemia 2 h						
GL $n=5$	61 1±2.3**	143.2± 8 6	10.5±1.3	9.2±1 6	91 1± 4 7*	−71 0±0 4
Sol $n=5$	33.5±2.7	61 0±10 4	6.6±1.3	3.5±0.5	63 4± 4.8	−69 0±1 4
Control 2 h						
GL, $n=4$	57 0±6 9	189.8±15.5	16 0±4 4	4 7±1 1	17.8± 2.2	−90.3±0.8
Sol $n=5$	40.5±4.2	135 6± 7 7	11 0±1.5	2.0±0.8	5.8± 0 6	−89 4±0.9

* $P<0.05$ ** $P<0.01$ GL vs. Sol.

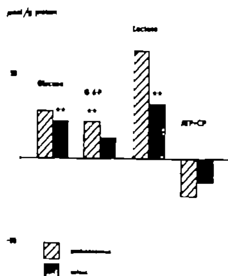


Fig. 1. Changes in the metabolite content of the soleus and gastrocnemius muscle induced by h of hemorrhagic shock. $P<0.01$ vs. initial value.

hile that of the soleus was slightly lower -87.2 ± 1.1 mV. In the nonischemic control limb of animals subjected to tourniquet ischemia, no significant alterations of the metabolite content or the membrane potential occurred during the 2 h experimental period (Table 1).

Hemorrhagic shock

The hemorrhage resulted in a rapid drop in arterial BP from an initial value of 126 ± 7 mmHg to below mmHg within a few min. An incomplete regain to about 70 mmHg took place during the following 30-60 min, whereafter a slow deterioration ensued. At 2 h the BP was 53 ± 7 mmHg. In Fig. 1 the shock-induced changes in the metabolite content of the soleus and gastrocnemius muscles are shown. The hemorrhage resulted in significantly increased levels of glucose, G 6-P and lactate in both muscles. There was a 3-fold increase in the glucose content both muscles after 2 h of shock. The G 6-P content was significantly higher after the same time period in the gastrocnemius (26.8 ± 3.7) than in the soleus muscle (14.7 ± 9). The lactate accumulation was also greater in the gastrocnemius than in the soleus, 82.7 ± 11.3 and 40.2 ± 5.7 respectively after h.

The shock period resulted in a significant reduction of the CP content ($P<0.05$ vs. initial value) and a significant increase in the ATP content ($P<0.05$ vs. initial value) of the gastrocnemius muscle. The corresponding changes in the soleus muscle were less pronounced and no significant changes in either ATP or CP occurred. The total high-energy phosphagen content (taken as the sum of ATP and CP) was not significantly reduced in any of the muscles. There was a successive decrease in the resting membrane potential of both muscles to $-81._\pm1.4$ mV in the gastrocnemius muscle ($P<0.01$ vs. initial value) and to -75.8 ± 1.4 mV in the soleus muscle ($P<0.01$ vs. initial value) after 2 h.

Tourniquet ischemia

In cats subjected to tourniquet ischemia the mean arterial BP was 142 ± 7 mmHg and no significant alterations from this level occurred during the h experimental period.

The metabolic changes induced by h of ischemia are shown in Fig. 2. Complete ischemia resulted in a significant depletion of glucose in both muscles. In the gastrocnemius muscle there was a small but significant increase in the G 6-P content, whereas there was no significant change in the G 6-P content of the soleus. The lactate content in-

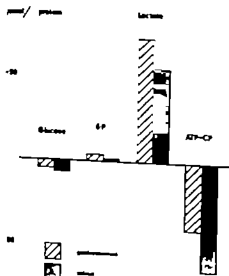


Fig. 2. Changes in the metabolite content of the soleus and gastrocnemius muscle induced by 2 h of tourniquet ischemia. $P<0.05$ ** $P<0.01$ vs. initial value.

Table 2 *Correlation between muscle metabolites and resting membrane potential during shock and tourniquet ischemia*

GL=lateral gastrocnemius muscle Sol=soleus muscle

	GL r	Sol r
ATP/−mV		
Shock	−0 41	−0.47
Ischemia	−0 46	+0 14
CP/−mV		
Shock	+0.58	+0.29
Ischemia	+0 61	+0 78
ATP+CP/−mV		
Shock	+0 46	+0 05
Ischemia	+0.55	+0 72
Lactate/−mV		
Shock	−0 82	−0 91
Ischemia	−0 97**	−0.91

$P<0\ 05$ $P<0\ 01$ $P<0\ 001$

creased significantly in both muscles In the soleus muscle the lactate accumulation was significantly higher after 2 h of tourniquet ischemia than after the same period of shock ($P<0\ 01$) while in the gastrocnemius muscle a similar level was reached under both conditions The 2 h of ischemia resulted in a 50% reduction of the CP content ($P<0\ 01$ vs initial value) in the soleus muscle The ATP content remained mainly unchanged *resulting in a 40% re*duction of the total high-energy phosphagen content (ATP+CP) In the gastrocnemius muscle the changes in ATP and CP were similar to those induced by hemorrhagic shock

Complete ischemia resulted in a rapid decrease in the resting membrane potential of both muscles which was significant after only 30 min −82.3±1 3 mV ($P<0\ 01$ vs initial value) and −82.3±1 4 ($P<0\ 05$ vs initial value) in the gastrocnemius and soleus respectively There was then a further continuous decrease and after 2 h the mean membrane potential of the gastrocnemius was −71 0±0 4 mV while that of the soleus muscle was slightly lower 69 0±1 4 mV

Regression analysis shows that there was a significant relationship between the tissue lactate content and the membrane potential in both muscles studied during shock as well as during ischemia (Table 2)

When shock and ischemia data are combined, the relationship −mV=92 38−0 18×lactate (r=0 812, $P<0\ 001$) was found for the gastrocnemius muscle and the relationship −mV=87.8−0 28×lactate (r=−0 902 $P<0\ 001$) for the soleus muscle. Comparison of the slopes of the two regression lines obtained fails to show any statistical difference between the slopes A significant relationship is also found between the CP content and the membrane potential of the soleus muscle during tourniquet *ischemia as well as between ATP+CP* and the membrane potential (Table 2)

DISCUSSION

During control conditions the high-energy phosphagen content as well as the G 6-P and lactate content were significantly higher in the gastrocnemius than in the soleus muscle This is in agreement with previous reports on metabolic differences between resting red" and white mammalian skeletal muscle (Ogata 1960 Beatty et al 1963) Studies on rats (Yonemura 1967) and guinea-pigs (Campion 1974) have shown that "red" muscle fibers have a considerably lower resting membrane potential than white" fibers In the present study on cats we were not able to demonstrate any significant difference in the resting membrane potential in the two muscles studied This might be due to differences in the measurement technique or to a species variation (Campion 1974)

The two models used in the present study were chosen because they made it possible to study the *metabolic response to hypoxia under two complete*ly different conditions During hemorrhagic shock differences in microvascular supply and blood flow distribution as well as differences in the reaction to circulating substances between the two types of muscle may modify their response to hypoxia. During tourniquet ischemia, on the other hand there is no blood flow and consequently no oxygen supply and a very limited diffusional transport making the muscle cells dependent on endogenous substrate.

The early metabolic reaction in skeletal muscle in hemorrhagic shock may be assumed to be due to the combined effects of tissue hypoxia and circulating substances e g catecholamines The increased blood levels of adrenaline during shock accelerate glycogenolysis in skeletal muscle (Belford & Feinleib 1964 Schumer & Erve 1975) The G 6-P levels were significantly higher in the gastrocnemius *muscle than in the soleus muscle after 2 h of shock* consistent with the known higher glycolytic ca ac-

of the former type of muscle (Ogata 1960 Beatty et al. 1963 Stubbs & Blanchaer 1965 Bocek et al 1966). Glucose is a mainly extracellular compound in skeletal muscle and a similar increase in the glucose content was observed in both muscles, reflecting an increase in blood glucose (Halmagyi et al. 1966).

The shock condition resulted in a shift to a more anaerobic metabolism in both muscles as evidenced by tissue lactate levels It has been shown that there is a good correlation between tissue lactate and tissue pH in skeletal muscle (Sahlin et al. 1975). The lactate accumulation during shock was more pronounced in the gastrocnemius muscle than in the soleus, and it may therefore be assumed that the tissue acidosis was more pronounced in the gastrocnemius muscle Furthermore the changes in ATP and CP observed in the gastrocnemius muscle during shock are compatible with a pH dependent shift in the ATP-CP equilibrium (Sahlin et al 1975) In the soleus muscle no changes in either ATP or CP occurred during shock. This confirms recent observations by Chaudry et al. (1976) on energy metabolism in rat soleus muscle during early shock. "Red" muscles are dependent during physiological conditions on an aerobic metabolism. It is therefore surprising, as indicated by the present results that the extent of metabolic impairment was less pronounced in the soleus muscle than in the gastrocnemius during shock. An early response to hemorrhage is a pronounced vasoconstriction in skeletal muscle due to an increased sympathetic discharge (Bond et al. 1969) The vascular beds of "red" muscles have been shown to be less sensitive to sympathetic stimulation than those of "white muscles (Folkow & Hudlicka 1968 Hilton et al 1970, Gray 1971). It may therefore be assumed that during shock, a redistribution of the blood flow within the skeletal muscle tissue takes place favouring "red" muscles at the expense of "white ones thereby initially maintaining a relatively high oxygen supply to "red" muscles and enhance clearance of waste products. The shorter diffusion distances in "red" muscles, due to a richer capillary supply and a smaller mean fiber diameter (Romanul 1965 Schmalbruch 1971) compared to "white muscles as well as the high myoglobin content (Reis & Wooten 1970) may also be important factors in this context.

In animals subjected to tourniquet ischemia there was no general sympatho-adrenal reaction, as evidenced by mainly unchanged metabolite and membrane potential levels in the non-ischemic control limb after h. This is in agreement with previous reports on the reaction to tourniquet ischemia in humans (Haljamäe & Enger 1975) and dogs (Enger et al 1978) In contrast to the situation during shock the total ischemia induced only small changes in the G 6-P content of both muscles, probably due to the absence of hormonal stimulation of glycogenolysis. The lactate accumulation was less pronounced in the soleus than in the gastrocnemius muscle during ischemia In the former muscle however a 40% reduction of the high-energy phosphagen content occurred whereas in the gastrocnemius the high-energy phosphagen content was mainly unchanged. During these extreme conditions of tissue anaerobiosis the glycolytic capacity of the soleus muscle was apparently insufficient to prevent energy depletion.

It has previously been shown that there is a decrease in the potassium content and an increase in the sodium content of single skeletal muscle cells during hemorrhagic shock (Haljamäe 1970). These electrolyte disturbances have repeatedly been shown to be reflected in a decrease in the resting membrane potential of skeletal muscle during shock (e g. Campion et al. 1969 Cunningham et al. 1971*b* Trunkey et al. 1973 Jennische et al. 1978) as well as during total ischemia (Arango et al. 1976, Enger et al. 1978). The reason for these changes in the membrane function during hypoxia is not known. The maintenance of the resting membrane potential is an energy-dependent process, and it may therefore be assumed that the depolarisation is due to an interference with normal cellular metabolism. However the alterations in the membrane potential during hypoxia probably do not result from energy depletion, since as recently shown by Gallaghan & Shires (1977) reduction of the membrane potential of skeletal muscle occurs in spite of normal ATP levels during shock. These results were confirmed in the present study

It also has been shown previously that there is a close relationship between tissue lactate content and the membrane potential in skeletal muscle during shock and during ischemia (Jennische et al. 1978*a* 1978*b*) In the present study as well a significant correlation was found between these two variables during shock as well as during ischemia in both muscles studied. The relation between the extent of metabolic impairment and the change in

membrane potential was most obvious in the soleus muscle In the gastrocnemius muscle the decrease of the membrane potential during tourniquet is chemia was somewhat more pronounced than would be expected from the simultaneous metabolic changes It seems that multiple factors affect the level of the membrane potential during hypoxia, and that these factors are different and/or of differ ent relative importance during the two hypoxic situations studied

The present data do not allow any conclusion about whether there is a causal relationship be tween the tissue lactate content and the membrane potential during hypoxia, or whether these are in-dependent but parallel phenomena. The good cor relation obtained however suggests that the in creased local hydrogen ion activity during hypoxia affects the cellular membrane either directly or indi rectly via effects on enzyme systems

On the basis of the present results it may be concluded that "red" muscles in accordance with their metabolic dependence on oxygen are more liable to ischemic damage during complete ischemia than "white" muscles whereas during a general hypoperfusion state such as hypovolemic shock "red" muscles appear to be protected from is chemic damage probably due to a redistribution of blood flow

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rostacyclin dependent coronary vasodilation in rabbit nd guinea pig hearts

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WENNMALM, Å Prostacyclin-dependent coronary vasodilation in rabbit and guinea pig hearts. Acta Physiol Scand 1979 106. 47 52. Received 6 Nov 1978 ISSN 0001-6772. Department of Clinical Physiology at Karolinska Institute Huddinge Hospital, Sweden.

Isolated hearts from rabbits or guinea pigs were perfused according to Langendorff and the coronary flow was recorded continuously In addition the rabbit heart transmyocardial effluent' content of platelet anti-aggregatory (prostacyclin-like PCLA) activity was assayed biologically at regular intervals Perfusion was performed with a solution gassed with 95% O_2 in CO_2, switching at intervals to a solution gassed with 12% O_2 and 5% CO_2 in N_2. Perfusion with a hypoxic solution elicited reproducible increases in coronary flow After pretreatment with indomethacin (5 10^{-6}M), this increase was completely abolished and in several cases it was reversed to a marked reduction in coronary flow The transmyocardial effluent contained, during perfusion with normoxic solution no detectable or only negligible amounts of PCLA. During hypoxia the efflux of PCLA into the transmyocardial effluent increased markedly This increase was completely abolished when indomethacin (5 10^{-6}M) was added to the solution perfusing the heart. The results strongly suggest that increased coronary vascular formation of prostacyclin plays key role in the coronary vasodilation induced by hypoxia in rabbit and guinea pig hearts.

Key ords Coronary flow guinea-pig heart, platelet aggregation, prostacyclin, rabbit heart

ie role of prostaglandins (PG) formed in the corory vasculature in the development of hypoxic peremia has been debated intensely in recent ars Initially it was reported that indomethacin or clofenamate cause a marked reduction in the sodilator response to ischemia and hypoxia in en-chest dogs (Kent et al 1973 Alexander et al. 75) These data were strongly supported by the servation of Afonso Bandow & Rowe (1974) that reduced amount of oxygen in the inhalation gas xture induced an increase in coronary blood flow at was considerably lowered by indomethacin. An creased formation of PGE- and PGA-like subnces in conjunction with coronary reactive peremia was also observed to follow 10 min of ronary artery occlusion (Kraemer Phernetton & ltis (1976). These authors, moreover reported re ced reactive hyperemia and abolished release of E- and PGA-like substances following pretreat ent with indomethacin. However contradictory sults have also been presented. Needleman et al (1975) reported that anoxia produces coronary vasodilation without causing release of PG-like substances and that indomethacin fails to abolish coronary vasodilation produced by ischemia, hypoxia or anoxia in isolated perfused rabbit hearts. Furthermore Owen et al. (1975) and Hintze & Kaley (1977) found that brief occlusions of coronary blood flow (10–20 s) in closed-chest mongrel dogs induce an increase in coronary blood flow that is not inhibited by indomethacin. In addition, it has been pointed out that the release of PG which follows cardiac ischemia is not concurrent with the vasodilation (Block et al 1975)

It appears from the above-mentioned studies that brief coronary occlusions in closed-chest dogs fail to elicit a PG-dependent coronary vasodilation, while in open-chest dogs and during more severe ischemia there is an increase in coronary flow that can be reduced by indomethacin. This observation suggests that moderate cardiac hypoxia does not stimulate PG synthesis whereas a more severe re

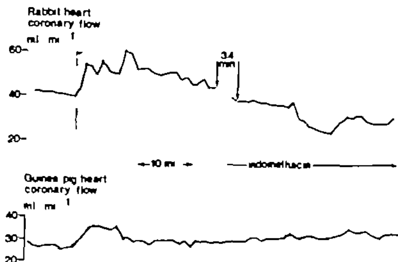


Fig 1 Effect of hypoxia on the coronary flow rate in rabbit and guinea-pig isolated hearts before and after addition of indomethacin (5×10^{-6} M) to the medium perfusing the hearts The striped areas represent the periods during which the hearts were perfused with a solution gassed with 1 % O_2 (instead of 95 % O_2 which was used otherwise)

duction in tissue pO_2 is efficient in this respect This assumption is supported by the recent demonstration (Kalsner 1977) that PG synthesis in isolated strips of bovine coronary arteries is accelerated by hypoxia unless the oxygen deprivation is so severe as to limit the availability for synthesis

The present study was therefore undertaken to investigate whether sustained moderate hypoxia in isolated guinea pig or rabbit hearts elicits a vasodilation in the coronary vasculature that is dependent on an intact cardiac formation of PG Analysis of PG efflux from the heart was focused on prostacyclin since this is the main PG formed in the heart (Isakson et al 1977 De Deckere et al 1977)

MATERIALS AND METHODS

a. Heart perfusion

Rabbits of mixed sexes and strains weighing from 2.5–4.5 kg or male guinea pigs weighing from 600–800 g, were used for the study The animals were killed by a blow on the head and exsanguinated via the carotid artery The hearts were prepared according to Langendorff and perfused at a constant pressure of 60 cm H_2O and 37°C with a solution containing (in mM) NaCl 137 KCl 2.7 $CaCl_2$ 1.8 $MgCl_2$ 1.0 $NaHCO_3$ 12 NaH_2PO_4 and glucose 5.6. The rabbit hearts were prepared in addition according to De Deckere et al (1977) which allows separate collection of coronary effluent (Q_{cv}) and transmyocardial interstitial effluent (Q_i)

The perfusion apparatus consisted of two flasks connected via a stop cock close to the aortic cannula. Both flasks contained Tyrode solution one aerated by a high oxygen gas mixture (5 % CO_2 in O_2) and the other by a low oxygen gas mixture (5 % CO_2 and 1 % O_2 in N_2). The low oxygen Tyrode solution will be referred to as the hypoxic solution and the high oxygen Tyrode solution as the normoxic solution

b *Coronary flow experiments*

Coronary flow was estimated by collecting and weighing the Q_{cv} effluent (rabbit hearts) or the total cardiac effluent (guinea-pig hearts) in consecutive 1 min periods.

After a 15 min equilibration period during which the heart was perfused by normoxic solution hypoxia was induced during 8 min by switching to perfusion with

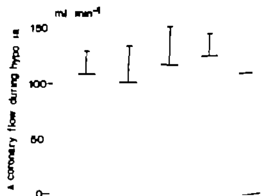


Fig 2 Increase in coronary flow (total flow-preceding basal flow) induced by five repeated 8 min. periods of hypoxia. The hypoxia periods were separated by 10 min intervals of perfusion with normoxic solution As seen from the fig repeated periods of hypoxia elicit flow increases of reproducible magnitude The data are presented as mean ± S E. The number of experiments is indicated at the bottom of each column.

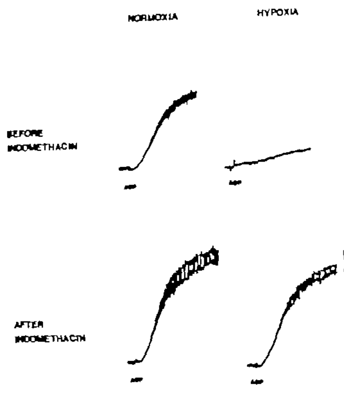


Fig 3. Typical platelet aggregation curves obtained by addition of 2.5 μg of ADP to a mixture of 0.50 ml human platelet rich plasma, 0.1 ml tris buffer and 0.5 ml transmyocardial (Q_t) effluent. Aggregation was monitored in a photometer and the curves display the increase in light transmission occurring when the platelets aggregate. As seen from the fig., Q_t effluent collected during normoxic perfusion did not contain anti-aggregatory activity while effluent collected during hypoxia was able to inhibit aggregation. Indomethacin abolished the efflux of anti-aggregatory activity induced by hypoxia.

...oxic solution. After that, perfusion was switched back ... normoxic solution, which was maintained for 20 min. ... the normoxic period, a new period of hypoxia was ... duced, followed by normoxia. After the two periods of ... poxia, indomethacin was added to both the normoxic ... of the hypoxic solution to produce a final concentration ... $5 \cdot 10^{-6}$ M. With indomethacin, periods of hypoxia, ... parated by normoxia, were induced as above. The ... onary flow was recorded during the entire experiment. ... some cases 3 to 5 hypoxia periods were induced with... ... addition of indomethacin. These experiments served ... controls.

... *Biological assessment of cardiac efflux ... prostacyclin-like activity*

... venous blood was obtained from donors who had not ... taken aspirin-like drugs for at least one week. It was ... collected in test tubes containing 1/10 vol of 0.13 M ... sodium citrate. Platelet rich plasma (PRP) was obtained by centrifuging the blood at 200 g for 15 min. Aggregation was induced by the addition of 2.5 μg of ADP (Sigma Chemicals) to 0.5 ml PRP in 0.1 ml 0.1 M tris buffer, pH 7.4. Aggregation was monitored in a Vitatron UC 200 photometer connected to a W + W 1100 ink recorder.

Rabbit heart transmyocardial effluent (Q_t) was collected during 1 min every 3–4 min during the expt. It was immediately diluted to a fixed volume, usually 1 ml, with Tyrode solution. The dilution was performed in order to compensate for the small differences that were observed in the efflux rate of Q_t. 0.5 ml of the Q_t effluent was added to the PRP-tris buffer mixture and at a standardized time after the end of collection, usually 60 sec, ADP was added and aggregation was monitored.

d. *Calculations*

The changes in coronary flow induced by hypoxia were assessed as follows. The change in flow during the hypoxia period and the following 5 min, compared to the

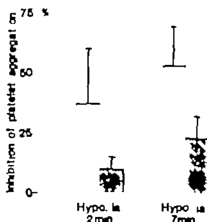


Fig 4 Inhibition of ADP-induced platelet aggregation (for calculation see text) induced by rabbit heart transmyocardial effluent (Q) collected after 2 and 7 min respectively of perfusion with hypoxic (1 % O) solution Open bars, no drug. Striped bars, indomethacin (5×10^{-6} M) in solution perfusing the heart. The data are based on 4 different rabbit heart perfusions and presented as mean±S E

basal flow preceding the hypoxia period was estimated graphically from the separate flow recordings. The efflux of CPLA was estimated from the separate aggregation recordings. The efflux is expressed as the change in the amplitude of the aggregation curve during the first 60 s in relation to control curves obtained with Q_i-effluents collected before the hypoxia period

Values are presented as mean±S E Student's *t* test for paired differences has been used when applicable

RESULTS

a. *Coronary flow*

The basal coronary flow of the rabbit hearts recorded after the initial period of equilibration with the perfusion system was 43±3 ml/min ($n=5$) This flow usually declined with time the reduction by the end of the expt usually being 30–50%

Changing the perfusion to a hypoxic solution induced an increase in the coronary flow (Fig 1) In some cases this increase was rapid and sustained while in others an initial peak increase was followed by a somewhat lower sustained rise In the former cases changing to a normoxic perfusion solution lowered the flow to the prehypoxia level within 1–2 min while in the latter cases a brief (3–5 min) increase in flow usually developed The mean increase in coronary flow during the hypoxia period and the first 5 min of the subsequent normoxia was 54±26 ml ($n=4$) The large S E. reflects the wide range of flow responses observed (15–170 ml)

Addition of indomethacin (5×10^{-6} M) to the perfusion solution did not change the basal coronary flow rate The response of the coronary flow to hypoxia was however affected inasmuch a reduction regularly materialized (Fig 1). The reduction during the hypoxia and the following min was 71±8 ml ($n=4$) The difference between the flow responses to hypoxia before and after indomethacin is significant ($P<0.01$)

In the control series in which repeated periods of hypoxia were induced in the absence of indomethacin the magnitude of the flow responses to hypoxia was unchanged (Fig 2) Thus no fading of the coronary flow response to hypoxia developed during repeated exposure to hypoxia.

In the guinea-pig hearts the basal coronary flow was 28±3 ml ($n=3$) This flow also decreased with time the reduction being of the same magnitude as that observed in the rabbit hearts. Perfusion with hypoxic Tyrode solution induced in all expts a marked and reproducible increase in coronary flow averaging 57±9 ml during the period of hypoxia and the following 5 min (Fig 1)

Indomethacin (5×10^{-6} M) did not affect the basal coronary flow in the guinea pig hearts but the response to hypoxia, in analogy with that in the rabbit hearts changed drastically In one experiment the increase in flow observed before indomethacin (… ml) was reversed by the drug to a decrease amounting to 140 ml and in the remaining expts. the increases in flow 43 and 73 ml respectively were completely inhibited (cf Fig. 1)

b *Outflow of prostacyclin-like activity*

In no case did transmyocardial effluent (Q_i), collected during perfusion of the heart with a normoxic solution before hypoxia, display PCLA as reflected by its anti-aggregatory capacity In contrast to this perfusion of the heart with hypoxic solution elicited the efflux of considerable amounts of PCLA into the Q_i-effluent (Fig 3) Anti-aggregatory activity was demonstrable in all cases after 7 min of hypoxia and in most cases already after 2 min (Fig 4) Perfusion of the heart with normoxic solution following hypoxia did not immediately prevent further efflux of PCLA In some cases the efflux observed during the first minutes of the normoxic period was of the same magnitude as during hypoxia The efflux of PCLA subsequently faded and 10 min after the hypoxia it could no longer be detected

In some expts the half-life of the anti-aggregat…

y activity was determined its range was found to 6-7½ min

Indomethacin (5×10⁻⁶ M) strongly inhibited the lux of PCLA elicited by hypoxia (Fig. 3 4) Inhibition was however not complete in all expts.

DISCUSSION

Perfusion of the rabbit heart with a low oxygen solution for 8 min consistently elicited an increase coronary flow that was abolished when perfusion was switched to normoxic solution. Control expts. early demonstrated that this vasodilation was of the same magnitude during repeated periods of hypoxia. Indomethacin an inhibitor of prostaglandin synthesis in tissues (Vane 1971) although it elicited no change in the basal coronary flow completely eliminated—and in some cases even reversed—the increase in flow caused by hypoxia. This observation strongly suggests that the coronary vasodilation observed was dependent on an intact formation of PG in the heart.

The main PG formed in vascular tissue is prostacyclin (Gryglewski et al 1976) Prostacyclin has also been shown to be the main arachidonic acid metabolite in the rabbit heart (DeDeckere et al. 1977 Isakson et al 1977) Prostacyclin has strong platelet anti-aggregatory properties and is unstable in aqueous solution (Gryglewski et al. 1976) these two properties distinguish prostacyclin from other cyclo-oxygenase products formed. In rabbit heart effluents collected during normoxia in the current expt no PCLA was observed but during perfusion with the hypoxia solution, considerable amounts of PCLA appeared in the effluent. The efflux of PCLA was counteracted by indomethacin, which suggests that the active principle was dependent on an intact function of cardiac cyclo-oxygenase The half-life of the activity was of the same order as that reported for prostacyclin (Gryglewski et al. 1976) It therefore seems reasonable to assume that the PCLA appearing in the cardiac effluent during hypoxia was prostacyclin The current data thus display a parallel increase in coronary flow and efflux of prostacyclin during hypoxia, as well as an inhibitory effect of indomethacin on both these parameters. The data thus support the hypothesis that coronary vasodilation induced by hypoxia is dependent on an intact cardiac formation of prostacyclin. As mentioned in the Introduction, it was recently demonstrated (Kalsner 1977) that PG synthesis is stimulated by moderate hypoxia, whereas complete anoxia seemed to counteract PG formation In the studies cited above in which the increase in coronary flow caused by coronary occlusion was found to be independent of indomethacin (Owen et al. 1975 Hintze & Kaley 1977) the reduction in tissue oxygen tension may possibly have been too severe to act as a stimulator of PG synthesis. That these studies nevertheless demonstrated an increase in coronary flow following coronary occlusion suggests that an accelerated formation of PG in the coronary vasculature is not the only factor involved in the vascular response to hypoxia or ischemia. In keeping with this it seems that increased coronary formation of PG may play a role in preserving not anoxic myocardial tissue (myocardial infarction) but tissue suffering from moderate hypoxia, as e g. the cardiac muscle in coronary insufficiency

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Local versus regional cerebral blood flow in the rat at high (hypoxia) and low (phenobarbital anesthesia) flow rates

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ABDUL-RAHMAN A. DAHLGREN N INGVAR, M REHNCRONA, S & SIESJÖ B K. Local versus regional cerebral blood flow in the rat at high (hypoxia) and low (phenobarbital anesthesia) flow rates. Acta Physiol Scand 1979 106: 53–60. Received 7 Nov 1978. ISSN 0001-6772. Laboratory for Experimental Brain Research, and Depts. of Anesthesia and of Neurosurgery University of Lund, Sweden.

Local cerebral blood flow (CBF) was measured in rats using an autoradiographic technique with ^{14}C-iodoantipyrine as diffusible tracer in situations with low normal and high flow rates (phenobarbital anesthesia, analgesia with 75% N_2O and hypoxia, respectively) A comparison of the results with previous data obtained in conscious rats (Sakurada et al. 1978) demonstrates that 75% N_2O moderately reduces local CBF in some but not all cortical and subcortical areas, that phenobarbital anesthesia reduces local CBF to between 30 and 65% of (conscious) control, and that pronounced hypoxia (arterial P_{O_2} about 25 mmHg) increases local CBF 3- to 4-fold. A comparison of the values obtained for cortical structures with those previously measured with a technique based on the Fick principle shows that the autoradiographic technique gives similar values at low and normal flow rates but that it moderately underestimates CBF at high flow rates, probably due to diffusion limitation.

Key words. Cerebral blood flow quantitative autoradiography phenobarbital anesthesia, N_2O analgesia, hypoxia

During recent years, methods have become available for the quantitative estimation of total regional, and local cerebral blood flow (CBF) in small laboratory animals like the rat. Available methods for estimating total and regional CBF are modifications of those previously developed for man or large experimental animals and are based on the procedures described by Kety & Schmidt (1948) and Lassen & Ingvar (1961) In the original application of the Kety & Schmidt (1948) principle to the rat, with ^{133}Xenon as the inert gas, cerebral venous blood was sampled from the superior sagittal sinus (Eklöf et al 1973 Norberg & Siesjö 1974) Since the sinus drains predominantly cortical areas the CBF values obtained should represent regional (cortical) blood flow A subsequent modification of the technique to include sampling from the retroglenoid vein (Nilsson 1974) gives values that probably represent total CBF (Nilsson & Siesjö 1976, Gjedde et al 1975) Recently the Lassen & Ingvar (1961) procedure was adopted for work in rats with injection of ^{133}Xenon into the internal carotid artery and external counting of the tissue clearance of inert gas (Hertz et al 1977). Since flow was calculated from the initial slope of the clearance curve the CBF values derived probably reflect predominantly gray matter flow

The advantage of techniques based on the Fick principle (Kety & Schmidt 1948) is that they lend themselves to quantitative estimation and to simultaneous derivation of cerebral metabolic rates while those employing external detection of tissue clearance of inert gas allow repeated measurements in the same animal However none of them give in-

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formation on local flow rates An ingenious technique for measuring local CBF was developed by Landau et al (1955 see also Freygang & Sokoloff 1958) The method is based on the fact that the rate of tissue uptake of an inert tracer that has an infinitely fast diffusion rate is a quantitative function of the blood flow Consequently it is possible to derive local CBF if one knows the history of the arterial tracer concentration the local tissue concentration of tracer and its tissue blood partition coefficient Since the tissue uptake can be measured autoradiographically the resolution of local blood flows is only limited by that of the autoradiographic techniques used Originally the tracer employed was ^{131}I trifluoroiodomethane Due to practical difficulties of synthesizing and using this compound the method was later modified for ^{14}C antipyrine (Reivich et al 1969) However subsequent work showed that antipyrine is diffusion-limited and thus leads to underestimation of CBF (Eklöf et al 1974 Eckman et al 1975) More satisfactory results were obtained with ^{14}C-ethanol (Eklöf et al 1974) but, due to its volatility this substance is impractical for use in autoradiography Recently Sakurada et al (1978) found that ^{14}C iodoantipyrine has a higher oil/water partition coefficient than antipyrine and that it gives local flow rates in cats and rats comparable to those obtained with trifluoroiodomethane

In the present experiments we applied the autoradiographic technique for local CBF (^{14}C iodoantipyrine) to normoxic and hypoxic rats maintained on 75% N_2O as well as to those anesthetized with 150 mg kg of phenobarbital Local CBF was thus obtained at low normal and increased flow rates Since regional (cortical) CBF has previously been measured in these three situations under similar experimental conditions the results provide novel information on the relationship between regional and local flow rates

METHODS

Operative techniques

The experiments were performed on 325–345 g male SPF rats of a Wistar strain (Møllegaard Avelslaboratorium Copenhagen) that had free access to rat pellets (Sanbolagen Malmö) and tap water until the day of operation. In two groups of animals anesthesia was induced with about 2% halothane delivered via a vaporizer (Dräger) When the animals were unresponsive they were tracheotomized and connected to a Starling-type respirator that delivered about 1% halothane and about 25% O_2 Immobilization was achieved with tubocurarine chloride (0.5 mg kg i v). Both femoral arteries and a femoral vein were cannulated One femoral artery was used for blood pressure recording with an electromanometer (Elema, Stockholm) and for anaerobic sampling of blood The other catheter was cut to a length of [illegible] and was later used for sampling of blood for ^{14}C iodoantipyrine measurements. The femoral vein catheter was used for infusing the tracer The animals were given heparin (100 I U i.v) When operative procedures were completed the halothane supply was discontinued and animals were maintained on 75% N_2O and 25% O_2 Ventilation was adjusted to give an arterial P_{CO_2} of 35-40 mmHg Body temperature as measured in the rectum was kept close to 37°C.

In the third group, anesthesia was induced with 150 mg kg of phenobarbital i.p When surgical anesthesia was obtained the animals were connected to the respirator and ventilated on about 25% O_2 Subsequently the animals were prepared as those maintained on 75% N_2O

Experimental groups

There were 3 experimental groups.

1 *Normoxic controls* ($n=6$) maintained on 75% N_2O and 25% O_2 Local CBF was measured about 30 min after the operative procedures had been completed.

2. *Hypoxia* ($n=6$) About 15 min after the end of the operation the oxygen content of the insufflated gas mixture was lowered so as to yield an arterial P_{O_2} of between 25 and 30 mmHg during 30 min. At the end of this period local CBF was measured

3 *Phenobarbital anesthesia* ($n=4$). Animals were maintained artificially ventilated for about 30 min before CBF was measured

In addition to these experimental groups 6 animals were used for measuring the partition coefficient for ^{14}C iodoantipyrine (see below)

Measurement of local CBF

Local CBF is calculated from the equation (Kety 1960, see also Sakurada et al 1978)

$$C_i(T) = \lambda K \int_0^T C_a e^{-K_i(T-t)} dt \qquad (1)$$

Where $C_i(T)$ is the tissue concentration of the tracer (^{14}C-iodoantipyrine) at a given time (T) after its introduction into the circulation, λ is the tissue: blood partition coefficient, C_a is the arterial concentration of tracer and K_i is a term which is defined as

$$K_i = mF/\lambda W \qquad (2)$$

Here F/W is the blood flow per unit mass of tissue and m is a factor between one and zero that expresses the extent of diffusion equilibrium With an infinitely fast diffusion, m is one while diffusion limitation is reflected in a value less than one Assuming that m is unity CBF (F/W) can be calculated if one knows the integral of C_a during time 0→T C_i at time T and λ. The partition coefficient (λ) for rat cerebral tissues was determined by Sakurada et al (1978) as being 0.80

Preparation and infusion of ^{14}C-iodoantipyrine ^{14}C iodoantipyrine (New England Nuclear) with a specific ac

ity of 50.4 mCi mmol⁻¹ was obtained in vials, each containing 0.25 mCi (2.5 ml) dissolved in ethanol. 0.5 ml of solution was evaporated in a stream of nitrogen gas and the tracer was re-dissolved in 0.5 or 1 ml of physiological saline. For infusing the tracer solution a 2 ml syringe was connected to an infusion apparatus and the solution was infused at a rate of 1 ml min⁻¹. Previous calculations have shown that the optimal infusion time (T, eq. 1) varies with the rate of blood flow (Eklöf et al. 74). Therefore, the infusion time chosen was 60 s in control animals (75% N_2O) and in those given phenobarbital, and 30 s in hypoxic animals.

Measurement of ¹⁴C activity in arterial blood. Arterial samples were drawn just before and during the period of infusion, every 5 s in two of the groups (control and phenobarbital) and every 3 s in the third (hypoxia). In order to avoid distortion of the arterial curve due to smearing, the catheter was short (see above), and no stopcock was used. In addition, one drop of blood was allowed to flow from the capillary between each sample. Between the sampling the catheter was closed by a pair of artery forceps. Samples were obtained in 40–50 µl glass capillaries drawn to fine tips at both ends. For filling the capillary one end was loosely fitted into the end of the catheter. A series of capillaries could be easily filled at 3 s intervals if the capillaries were attached (in parallel) to a wooden tongue spatula, which was laterally shifted between each sample. To count the ¹⁴C activity an aliquot of blood was introduced into scintillation vials. A reproducible volume was obtained by attaching the capillary to Hamilton syringes with fixed adapters set to deliver 30.0 µl. The vials contained 0.8 ml Soluene® and propanol in the ratio 1 : 1 (v/v). To the vials were then added 0.2 ml of 30% (w/v) hydrogen peroxide solution and 7.5 ml Instagel®. The samples were counted in a Nuclear Chicago liquid scintillation counter. Regularly the efficiency was calculated by means of an external standard but, occasionally, this was checked by adding an internal standard (¹⁴C hexadecane, Amersham) to the vials before recounting. Values thus obtained were used for calculation of local blood flow (see below).

Measurement of ¹⁴C activity in tissue. At the end of the 30 or 60 s infusion period, the animal was decapitated. The brain was then rapidly excised from the skull and frozen (within 2–3 min) in Freon 12 chilled to −50° to −70°C with liquid nitrogen. The frozen brain was then stored in plastic bags at −80°C until sectioned. Using a refrigerated box (−22°C) the brains were embedded in sodium carboxymethyl cellulose and fixed to object holders used with a Leitz Wetzlar sledge microtome. This microtome, placed in a deep freezer at −18 to −21°C, was used to prepare brain sections of 20 µm thickness. A series of 3 to 5 consecutive sections taken every 0.2 mm throughout the brain was used for autoradiography. Each section was picked up on a glass cover slip, dried on a hot plate at 60°C for 5 min and placed in an X-ray cassette. A set of methyl methacrylate standards (Amersham/Searle or New England Nuclear) was also placed in the cassette. The autoradiographs of the brain sections and the standards were prepared in the X-ray cassette with Kodak film, Type SB-5, 8 × 10 inches. After an exposure time of 7 days the films were developed according to the instructions supplied with the film. A calibration curve relating optical density of the film and the tissue ¹⁴C activities was determined for each film by densitometric measurement. Local tissue ¹⁴C activities in various gray matter structures were then determined from the calibration curve and the optical densities of areas representing various brain structures. Densitometric measurements were made using a transmission densitometer (Macbeth Color and Photometry Division, model TC 504) with an aperture of 1 mm. With the resolution thus obtained diffusion artefacts due to delay in freezing are negligible.

Measurement of tissue–blood partition coefficient. In order to validate the procedures used, the tissue–blood partition coefficient for ¹⁴C-iodoantipyrine was determined in 6 animals, two of which were made hypercapnic (Pa_{CO_2} about 80 mmHg) to increase CBF. In all the tissue activity was determined by both autoradiography and by liquid scintillation counting. The animals were initially prepared as were the control (75% N_2O) but with a brachial instead of a femoral catheter. To minimize metabolic degradation of the tracer the renal arteries and veins, the portal vein, the hepatic and superior mesenteric arteries, and the abdominal aorta (proximal to the coeliac artery) were ligated. A 0.5 ml solution containing 50 µCi ¹⁴C-iodoantipyrine was then injected i.v. and 1 h was allowed for brain-blood equilibration. Blood samples were taken every 15 min and just after the last sample the animal was decapitated. The brains were divided in the midline. One half was taken for autoradiography as described above while the other half was dissected in four portions (frontal, parietal and occipital cortex, as well as cerebellum). These portions (80–90 mg each) were weighed on a torsion balance at −22°C and taken for scintillation counting, using techniques similar to those used for blood. The tissue–blood partition coefficient was calculated separately from the tissue concentration obtained by autoradiography and liquid scintillation counting.

Calculations

CBF was calculated from eqs. 1 and 2, using a Wang desk computer. Statistical differences were calculated using Student's *t*-test.

RESULTS

Brain–blood partition coefficient (λ). Determination of λ requires that the blood tracer concentration is kept constant for a sufficient time to allow diffusion equilibrium to occur between blood and brain. Since the ¹⁴C activities varied between animals the activities at 15, 30 and 45 min were calculated in percent of the 60 min value. Fig. 1 shows that tracer concentrations in blood were constant for at least 30 min before tissue was sampled. Table 1 gives the partition coefficients obtained. Since there were no differences between normocapnic and hypercapnic animals the values for all six ani-

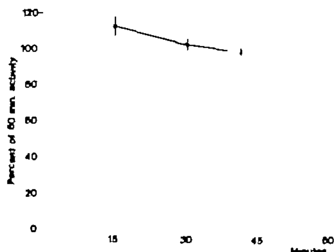


Fig 1 Stability of ¹⁴C-iodoantipyrine activity in arterial blood in experiments for determination of brain blood partition coefficient In each rat the values at 15 30 and 45 min were expressed in per cent of the 60 min values The values given are means ± S E

Table 1 *Brain tissue blood partition coeffi[illegible] for ¹⁴C iodoantipyrine*

The values are means ± S E. for groups of 6 animals

Region	Measured by autoradiography	Measured by liquid scintillation counting
Frontal	0 78±0 01	0 78±0 01
Parietal	0 79±0.01	0.79±0 02
Occipital	0 80±0 01	0.79±0 01
Cerebellum	0.80±0 01	0 79±0 01

mals were pooled The values obtained (grand mean 0 79) were in excellent agreement with those published by Sakurada et al (1978) The results show that similar values for λ were obtained using either autoradiography or liquid scintillation count ing and that identical values were obtained in different cortical and subcortical regions

In the experiments designed to determine the tis sue blood partition coefficient the activity of the tracer is homogeneously distributed in the tissue *Thus the brains are suited for controlling the reproducibility* of densitometric readings between consecutive sections When sectioning the 6 brains 20 series of 5 consecutive sections from each brain were applied to the X ray film and densitometric readings were obtained from the same region in the consecutive sections In all brains the coefficient of variation (expressed as the standard deviation in per cent of the mean) for the measured optical densities was below 6 5% *In view of this reproducibility* subsequent determinations of optical density in any one structure were made by taking the mean of 5–6 readings from 3 consecutive sections. [illegible] this procedure the accuracy in determining [illegible] activity should be lower than 5%

Local CBF in control and experimental gro[illegible] In the present study control conditions were [illegible] sumed to prevail in animals maintained on 7[illegible] N O and 25% O_2 while phenobarbital anesthe[illegible] and hypoxia (75% N_2O) were employed to prod[illegible] states with low and high flow rates, respective[illegible] Table 2 gives body temperature and mean arter[illegible] blood pressure as well as arterial P_{O_2} P_{CO_2} [illegible] pH The results show that body temperature [illegible] similar in all three groups that animals in phenob[illegible] bital anesthesia had reduced blood pressure but Pa_{CO_2} *similar to the control animals* and that [illegible] poxia was accompanied by reductions in bl[illegible] *pressure* P_{CO} and pH

Local CBF values in 1? different brain structur[illegible] are given in Table 3 In general phenobarb[illegible] anesthesia reduced CBF markedly in most regi[illegible] studied while hypoxia was accompanied by pr[illegible] nounced hyperemia. Details of results will be di[illegible] cussed below

DISCUSSION

The present results provide information on [illegible] major points (*a*) the influence of nitrous ox[illegible]

Table 2 *Physiological parameters in experimental groups*

The values are means±S E. MABP=mean arterial blood pressure

Experimental group	Body temp (°C)	MABP (mmHg)	Pa_{O_2} (mmHg)	Pa_{CO_2} (mmHg)	pH
Phenobarbitone (=4)	36 6±0 1	115±6	108±1	37.5±0.2	7 41±0 02
Control (*n*=6)	37 0±0.2	14 ±4	103±2	37 0±0 7	7 42±0 01
Hypoxia 30' (=6)	36 5±0 1	1?0±4	*6 9±1.2*	?9 6±2..	7 24±0 02

ble 3 *Local CBF (ml g^{-1} min) in normoxic rats during either N_2O-analgesia ("control") or phenobarbital anesthesia as well as in animals made hypoxic for 30 min during N_2O analgesia*

Values are means±S E All values in phenobarbital and hypoxic groups were statistically different from control (p<0.01)

Structures	Phenobarbital (=4)	Control (=6)	Hypoxia 30' (6)
Superficial cerebral structures			
Cortex			
Sensorimotor	0 63±0 02	1 33±0 08	4.89±0 62
Auditory	0 62±0 02	1 94±0.25	4 80±0 43
Visual	0.50±0.03	1.–1 _0 07	3.32±0 44
Parietal	0 61±0 04	1.22±0 07	4 60±0 47
Frontal	0.54±0 04	1 11±0.07	4 02±0.34
Deep cerebral structures			
Thalamus	0.58±0 02	1.08±0 06	4 22_0 49
Hypothalamus	0.50±0.03	0 89±0 03	3.57±0.39
Hippocampus	0 48±0 04	0.91±0.04	3 10±0.39
Caudate-Putamen	0.51±0 04	1 19±0 10	3 65±0.34
Mid-brain and pons			
Inferior Colliculus	0.77±0 05	2.02±0 20	5 05±0.33
Pontine gray	0 67±0 03	0.97±0 03	3.65±0.44
Cerebellum			
Cerebellar cortex	0.63±0 06	0.91±0 05	3 46±0.5?

analgesia on local CBF (*b*) the corresponding influence of phenobarbital anesthesia, (*c*) local CBF in hypoxia, and (*d*) the relationship between local and regional CBF When discussing the last point we will consider the possibility that ^{14}C-iodoantipyrine shows diffusion limitation at high flow rates.

( ) The influence of nitrous oxide analgesia on local CBF Assuming that the anesthetized, moderately restrained rats used by Sakurada et al. (1978) are comparable to those of the present study and representative of the conscious state, one can use the present results to evaluate the influence of 75% N_2O on local CBF Although previous results in man are somewhat variable (see Smith & Wollman 1972) both these and the results of animal experiments (Theye & Michenfelder 1968 Carlsson et al. 1976) indicate that nitrous oxide has small effects on CBF

As Fig 2 shows, the present data suggest that 75% N_2O does not alter CBF in some subcortical and cortical structures (e g. hypothalamus) but that it reduces flow by 20–30% in some others (sensorimotor and parietal cortex, thalamus). Somewhat unexpectedly the high flow rates observed in structures associated with hearing (auditory cortex and colliculus inferior see Sakurada et al. 1978) were also observed in the present N_2O-anesthetized animals. It must be tentatively concluded that activity in such pathways is minimally suppressed by nitrous oxide

(b) The influence of phenobarbital anesthesia on local CBF Barbiturate anesthesia is known to reduce overall CBF (see Smith & Wollman 1972). Previously it has been shown that "light thiopental anesthesia (disappearance of corneal reflex) is accompanied by a lowering of local CBF predominantly in cortical and gray matter structures in the cat brain i.e in areas that have a high flow in the conscious state (Freygang & Sokoloff 1958) As a result regional differences in flow become less pronounced. In the present expts local flow rates are representative of deep ("surgical") anesthesia. The results (Fig. 3) are qualitatively similar to those reported previously in that barbiturate anesthesia had a pronounced influence on CBF in cortical and other gray matter structures. If the comparison is made to the conscious state (Sakurada et al. 1978) some local flow rates were reduced to about 30% of control. In other areas notably brain stem structures the reduction was much less pronounced.

( ) Local CBF in hypoxia There are many previous studies of total and regional CBF in hypoxia

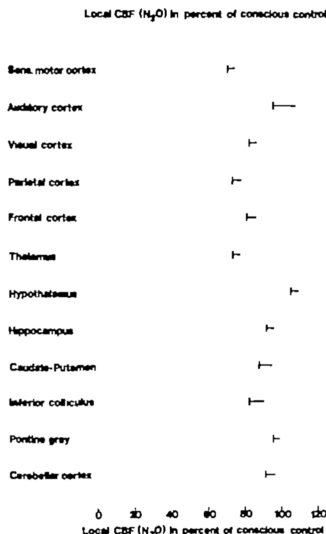


Fig 2 Local CBF in animals maintained on 75% N_2O and 25% O_2. The values (means ± S E.) are given in per cent of those of Sakurada et al (1978)

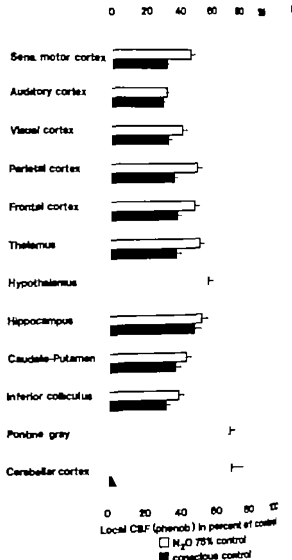
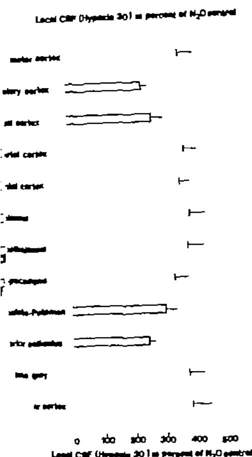


Fig 3 The influence of phenobarbital anesthesia on local CBF in the rat as compared with either N_2O-analgesia (present material) or with conscious rats (Sakurada et al. (1978) The values are means ± S.E.

which show that CBF increases in proportion to the reduction in Pa_{O} with maximal increases to 400–600% of control (for literature see Berntman et al 1978) Local CBF was determined by Freygang & Sokoloff (1958) in conscious cats exposed to a very moderate degree of hypoxia (10% O_2) The mean increase in CBF was 92% in gray matter and 65% in white matter In the present material (Fig. 4) the mean increases in flow varied between about 400% (parietal cortex thalamus and hypothalamus) to about 250% (auditory cortex and inferior colliculus) of control The relative increase in flow showed no obvious relationship to the control values For example, although some cortical structures (frontal parietal and sensorimotor) showed a 3 5- to 4-fold increase in flow others (auditory and visual) increased their CBF only 2.5- to ? 8-fold It seems apparent that hypoxia does not increase local CBF uniformly

(d) The relationship between local and regional CBF In all three situations studied presently regional CBF has been measured previously using ^{133}Xenon modification of the Kety & Schmidt (1948) technique Since venous samples for xenon determination were obtained from the superior sagittal sinus it can be assumed that the samples were representative of flow from mainly frontal and parietal cortical areas In order to allow a comparison to these regional flow rates the present results for frontal and parietal cortex were pooled (Fig. 9 The results show that there was a very good correspondence at low and normal flow rates but the



4 The influence of arterial hypoxia of 30 min duration local CBF in the rat. The values (means ± S.E.) given in per cent of N_2O control.

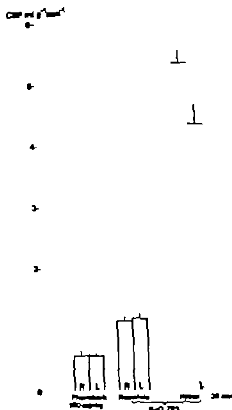


Fig. 5. A comparison between regional and local CBF at normal (N_2O analgesia), reduced (phenobarbital anaesthesia) and increased (arterial hypoxia) flow rates. The regional (cortical) CBF values were obtained from the following sources: Phenobarbital anaesthesia (Nilsson & Siesjö 1975), normoxia and hypoxia under N_2O (Berntman et al. 1978).

autoradiographically determined flow rates appeared somewhat lower than the regional ones at high flow rates. Since similar (unpublished) results have been obtained in hypercapnia we conclude that, at high flow rates, the tracer employed shows some diffusion limitation. This conclusion is in agreement with previous results (Eklof et al. 1974) which showed that none of the 4 substances tested, which included ^{133}Xenon and ^{14}C-ethanol fulfils the requirement of an "infinitely" diffusible tracer that can be used for the quantitative assessment of very high flow rates in the brain. Thus although ^{14}C-iodoantipyrine in many respects is an ideal tracer for measurements of local CBF values, values obtained at high flow rates must be corrected for a certain degree of diffusion limitation.

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The effect of vasodilatation and sympathetic nerve activation on net water absorption in the cat's small intestine

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BRUNSSON I EKLUND S JODAL, M., LUNDGREN O & SJÖVALL, H. The effect of vasodilatation and sympathetic nerve activation on net water absorption in the cat's small intestine. Acta Physiol Scand 1979 106. 61–68. Received 7 Nov 1978. ISSN 0001-6772. Department of Physiology University of Göteborg, Sweden.

The rate of net water uptake from the feline small intestine has been investigated during control conditions, during graded infusions of the vasodilator drug isopropylnoradrenaline and during electrical stimulation of the regional sympathetic nerve fibres to the gut. Net water absorption rate was largely unaffected by intestinal vasodilatation. The fraction of the absorbate transported via the lymphatics remained also constant at 20–40% of the total absorption regardless of blood flow rate. Stimulating the sympathetic nerve fibres to the small intestine increased, however net water absorption rate. The increase was particularly pronounced when blood pressure was kept constant during the period of stimulation. The absorption rate was on an average almost doubled at stimulation frequency of 8 Hz during constant pressure conditions. The mechanism(s) explaining this nervous control of water absorption are tentatively discussed.

Key words: Small intestine, water transport, intestinal blood flow sympathetic nerves adrenergic receptors

was observed already by Claude Bernard (1859) at removal of the solar ganglia induced a produc on of fluid in the intestines. Similar observations ave been made repeatedly by a number of workers or ref. see Florey et al 1941) but this effect was ot ascribed to a sympathetic denervation of the gut ntil the work by Wright and coworkers (1940). Most of the subsequent work performed with refer nce to the sympathetic control of intestinal water nd electrolyte absorption has been done using drenergic drugs such as noradrenaline Thus, Field McColl (1973 in vitro) and Hubel (1976 in vivo) udied the effects of adrenergic agents and con uded that the stimulation of α-adrenergic re ptors in the gut enhanced Na, Cl and water bsorption. It is however difficult to decide from ese experiments, if the observed effects were btained at "physiological" blood concentrations of oradrenaline. At the neuroeffector site of the ympathetic nerve fibres very high noradrenaline concentrations are produced by the release from the nerve varicosities. However anatomical studies have revealed only few sympathetic fibres close to the villous epithelium (Norberg 1964 Jacobowitz 1965 Silva et al 1971 Ahlman et al 1973) the site of the intestinal absorption

The present study was undertaken to study the importance of the sympathetic nervous fibres for water and electrolyte absorption during more "physiological" conditions The effects on absorption of direct electrical stimulation of the regional sympathetic nerves was investigated in vivo in cats with a technique that made it possible to study continuously the net water absorption. A pharmacological analysis of the observed nervous effect on net water transport was also carried out. Furthermore the effects of vasodilatation on net water transport were also determined.

A preliminary report of this paper has been published (Brunsson et al. 1976)

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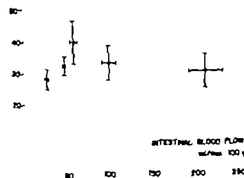
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2 The fraction of lymphatic net water absorption e in per cent of total when varying intestinal blood flow graded ... infusions of isopropylnoradrenaline. Each ... represents the mean value of 5–9 observations. Bars ... note ±S.E

...he effects of sympathetic nervous ...timulation ...d noradrenaline infusion on int...stinal ...t ...ater absorption

3 summarizes the results in all experiments of ...ectrical stimulation of the splanchnic nerves. The ...timulation frequency was set at 2, 4 or 8 Hz. Con...comitant with an increase of blood pressure and ...ecrease of total intestinal blood flow an increase ...f net water uptake from the gut was noticed

In Fig. 4 the results of Fig. 3 have been divided ...nto 2 groups. In one group (left panel) blood flow ...mained more or less at the control level during the ...riod of nervous stimulation due to an increase of ...terial blood pressure. In another group (right ...anel) arterial blood pressure was kept rather con...ant and intestinal blood flow was reduced during ...e sympathetic nervous activation. Such a ...emodynamic situation was in some of the experi...ments accomplished by allowing the animal to bleed ...nto a syringe. Comparing the two panels of Fig. 4 ...t is noticed that the nervous effects on watertrans...ort was most pronounced when blood flow de...creased. This is most clearly demonstrated at a ...timulation rate of 8 Hz, as is also illustrated in Fig. ... In this Fig. the relative increase of net water ...ansport is plotted vs. rate of nervous stimulation. In several experiments the period of nervous ...imulation was prolonged for 30 min or more. The ...crease of net water transport rate was maintained ...roughout the stimulation period as illustrated in ...ig. 6

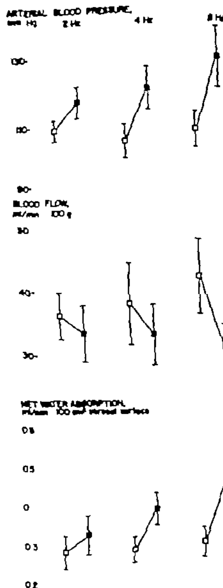


Fig. 3 The effects of electrical stimulation of the splanchnic nerves on arterial blood pressure, intestinal blood flow and intestinal net water absorption rate. The open symbols represent the control values and the filled ones the observations made while stimulating the nerve fibres. The results reported for 2 Hz were recorded in 10 experiments and the observations reported for the two other stimulations frequencies were made in 12 experiments. Bars indicate ±S.E

In 9 expts. a pharmacological analysis of the observed sympathetic nervous effects on water transport was performed. The administration of an α-adrenergic blocking agent (phentolamine 1–4 mg/kg b wt.) completely abolished the nervously induced

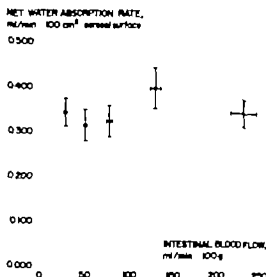


Fig 1 The relationship between rates of intestinal blood flow and net water absorption. Intestinal blood flow was increased by means of close i.a. infusions of graded doses of isopropylnoradrenaline Each point represents the mean value of 9–15 observations Bars denote ±S E

METHODS

Operative and experimental procedures

The experiments were performed on cats anesthetized i v with chloralose (50 mg/kg b wt.) after induction with ether The animals had been deprived of food for 4 h and had no obvious signs of intestinal diseases

The operative procedures as well as the recordings of blood flow and of net water absorption were similar to those described by Jodal et al. (1975) which should be consulted for details Briefly total venous outflow from one or two sympathetically denervated jejunal segments was recorded by a drop recording unit operating an ordinate writer In some experiments the intestinal segment was enclosed in a triangular perspex plethysmograph to record continuously the changes of tissue volume Mean arterial blood pressure was measured from the left femoral artery by a pressure transducer (Statham P23 AC)

The splanchnic nerve fibres were severed in all experiments In order to be able to stimulate electrically the sympathetic nervous outflow to the small bowel at the preganglionic level the distal ends of the divided splanchnic nerves were in some experiments placed on silver ring electrodes. Square wave stimuli were delivered from a Grass stimulator (model S5) and the nerves were stimulated at 5-7 V 5 ms and 2–16 Hz. The left adrenal gland was denervated and the right one was excluded from the circulation by ligatures.

Recording of net fluid absorption rate

Intestinal net water transport continuously measured with a recirculating system that was coupled to the lumen of the intestinal segments and contained a large reservoir (volume 1000 ml) to minimize recirculation. The volume change of the system was recorded with a volume tr ducer connected via a T-tube to the perfusion syste Provided no motility occurred the recorded chang volume reflected net water absorption or secretion (Jodal et al 1975) The intraluminal pressure of the outl end of the segment was kept at 1 cm H_2O and the intest segment was perfused at a constant rate of about 1 ml/ by means of a roller pump (Model mp-4 Ismatec Zürich, Switzerland) The temperature of the perfus entering the segment was continuously monitored wi thermocouple thermometer (Electrolab Copenhagen) kept at 38°C by a heating pad

Calculations

The weight of the intestinal segment and its lymph as well as the serosal surface area of the intestine w determined after each experiment. Blood flow rate expressed per 100 g intestinal tissue and net water upt per 100 cm² serosal surface area. The fraction of net wa uptake absorbed via the lymphatics was estimated as scribed in the paper by Jodal et al. (1975) When eval ing the effect of sympathetic nervous activation on recorded parameters the control values were calcul as the mean value of that recorded before and after nervous stimulation period

Solution

The lumen of the intestinal segments were perfused wi modified Krebs-Henseleit solution containing (mmol NaCl 122 KCl 4 7 KH_2PO 1.2 $NaHCO_3$ 25 $MgCl_2$ H_2O 1.2, $CaCl_2$ 2.5 glucose 30. The osmolality of t solution was about 310 mOsm/kg H_2O

Drugs

The following drugs were used, isopropylnoradrena noradrenaline phentolamine (Regitin® CIBA-Geig propanolol (Inderal® ICI) The animals were routi given atropine i v (0.5–1 mg/kg b.wt) at the sta the experiment.

RESULTS

Intestinal water absorption during intestinal vasodilatation

Intestinal net water transport was studied dur intestinal hyperemia produced by graded infusi of isopropylnoradrenaline The results of these e periments are illustrated in Fig. 1 It is clear that absorption rate of water was largely the same gardless of the rate of total intestinal blood flow

The experimental method used in this study m it also possible to estimate the fraction of total water absorption absorbed via the lymphatics w varying total intestinal blood flow Fig. 2 su marizes the results. The relative rate of lymph water absorption was not influenced by changes total intestinal blood flow

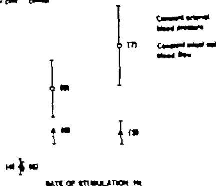


Fig 5 The relative increase of net water absorption rate observed when stimulating electrically the sympathetic nerve fibres to the intestine at 4 and 8 Hz. The observations are as in Fig 4 divided in experiments performed at constant flow or constant pressure conditions. Number within parentheses denote number of observations. Bars indicate ±S E

...point, did not significantly alter rate of water absorption. Similar observations were reported by Winne (1970) This author demonstrated in the rat that net water absorption from an isotonic Ringer solution was not much influenced by rate of blood flow when it was varied from high to low values passively by alterations of perfusion pressure In fact, net water uptake from a glucose Ringer solution even decreased when augmenting blood flow

Winne also followed the unidirectional fluxes of water across the intestinal epithelium and showed that fluxes in both directions increased upon vasodilatation.

The increase of intestinal blood flow produced by a vasodilator drug may influence a number of physiological parameters of importance for water transport across the intestinal epithelium, the net result being a largely unaltered rate of absorption (Fig. 1) First an increased number of villous capillaries are being perfused upon vasodilatation due to the opening up of pre-capillary sphincters increasing the capillary surface area for absorption and secretion of fluid (Folkow et al 1963 Svanvik 1973) Second, the increase of blood flow reflects predominantly a relaxation of precapillary resistance vessels, which in turn, decreases the pre to postcapillary resistance ratio Such a change leads to an increased mean capillary hydrostatic pressure which may produce intestinal secretion. Third, increasing intestinal blood flow augments linear rate of flow in the hairpin loops of the intestinal countercurrent multiplier which tends to decrease the interstitial hyperosmolality in the villi (Haljamäe et al 1973 Jodal et al. 1978) This tissue compartment has been inferred to exert an osmotic force of great importance for intestinal water absorption. Hence a decrease of villous osmolality may decrease the lumen to tissue flux of water Fourth the drug itself may influence the active transport processes of the enterocyte In case of

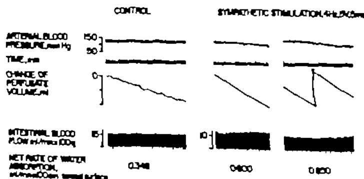


Fig 6 An experiment demonstrating the effect of electrical stimulation of the splanchnic nerves on blood pressure intestinal blood flow and net rate of water absorption. The rate of water uptake was calculated from the continuous decrease of the perfusate volume depicted in the middle of the Fig Asterisk denotes the addition of 1 ml perfusate to the volume transducer of the perfusion system. The times indicated during sympathetic nerve stimulation indicate times after start of stimulation

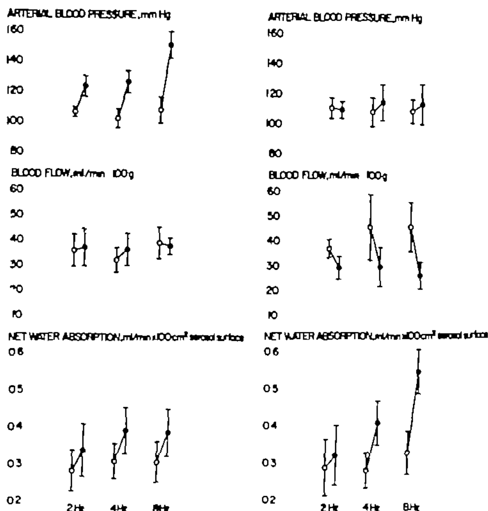


Fig 4 The results of Fig 3 divided into two groups The experiments in which blood flow remained fairly constant during the period of sympathetic nerve stimulations are shown on the left panel while those experiments in which blood pressure was maintained at a rather constant level are illustrated in the right panel. For numbers of observations see Fig 5 Bars denote ±S E.

increase of water transport. Concomitantly the sympathetically induced nervous vasoconstriction disappeared In fact in 4 out of 5 expts an intestinal vasodilatation was seen upon electrical stimulation of the sympathetic fibres to the gut In two of these experiments a doubling of blood flow was observed

An attempt to study the effect of β-blockade on the sympathetic influence on intestinal net water absorption was performed in 5 expts by giving propanolol i v or close i a. (1–4 mg/kg b wt) When giving this adrenergic blocking agent in such amounts that it abolished the effect of i.a injected isopropylnoradrenaline intestinal motility was started This made it impossible to investigate the effect of the β-blocker with the method used in this study

In 4 experiments noradrenaline was infused close i.a into the superior mesenteric artery At an infusion rate of about 1 nmol/min (0 2 μg/min) a [illegible] increase of net rate of fluid absorption was observed Such a comparatively low infusion rate produced a 5–25 % decrease of intestinal blood flow while no effect on blood pressure was apparent.

DISCUSSION

This study of net fluid transport in the small intestine consists of two parts In one series of expts the effects of inducing an intestinal vasodilatation were studied and in another the sympathetic nervous effects were investigated These two parts will be discussed separately below

Intestinal fluid transport during vasodilatation Increasing intestinal blood flow by close i a infusions of isopropylnoradrenaline a β-adrenergic

Third, sympathetic nerves may via a ganglionic site of action inhibit release of a neurotransmitter which inhibits water uptake. It has for example recently been proposed that vasoactive intestinal polypeptide (VIP) is a neurotransmitter in the gastrointestinal tract (Fahrenkrug et al. 1978a, b). VIP is known to produce a secretory state in the small bowel, and the release of this peptide from the gastrointestinal tract is decreased upon sympathetic nervous stimulation (Fahrenkrug et al. 1978a). Another possible transmitter substance with a similar action on water transport is acetylcholine (see Hubel 1976). However, the experiments of the present study were performed on atropinized animals.

Finally, a short comment on the observation that stimulation of the sympathetic nerve fibres to the gut produced an intestinal vasodilatation after α-adrenergic receptor blockade. Similar observations have been reported by Dale (1913), Bülbring & Burn (1936) and Ross (1973). The functional significance of this non-adrenergic, non-cholinergic vascular effect is not known.

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isopropylnoradrenaline no receptor for this drug has been demonstrated on the enterocyte (Field & McColl 1973)

The present method made it possible to estimate the rate of net water absorption occurring via the lymphatics As is shown in Fig 2 20–40% of the total amount of water absorbed left the intestine via the lymph vessels Even higher values have been reported by Simmonds (1954) and by Barrowman and Roberts (1967) These observations may seem surprising in view of the fact that water is a small molecule passing easily through the endothelium of blood capillaries and taking into account that total intestinal blood flow in the "resting situation can be calculated to be about 500 times greater than intestinal lymph flow (Wilson 1962) As has been discussed in two previous reports from this laboratory (Haljamäe et al 1973 Jodal et al 1978) the lymphatic absorption of water is probably explained by the presence of a countercurrent multiplier in the intestinal villi According to this hypothesis a gradient of interstitial pressures is established along the villus as a consequence of the villous hyperosmolality created by the countercurrent multiplier This hydrostatic pressure gradient provides the pressure head necessary for the flow of water along the central lacteal in the villus

Intestinal fluid transport during sympathetic nervous stimulation The results of the present study suggest that activation of the sympathetic fibres to the gut increases net water uptake via an α adrenergic mechanism This observation is in accordance with previous reports where exogenously supplied α-receptor agonists were used (Wright et al 1940 Field & McColl 1973 Hubel 1976 Levens et al 1977) Thus Field & McColl (1973) and Hubel (1976) demonstrated during in vitro and in vivo conditions respectively that noradrenaline increased net water uptake probably via an effect on net uptake of Na and Cl

It might be argued that the observed decrease of the recirculating volume observed in this study may not necessarily reflect an augmented rate of intestinal absorption but merely a relaxation of the intestinal smooth muscles i e the nervous effect on motility rather than on fluid transport It is known that activation of the sympathetic fibres produce a rapid and well maintained inhibition of intestinal motility (Kock 1954 Kewenter 1965) mainly via inhibition of intramural excitatory neurones (Kewenter 1965 Jansson & Martinson 1966) However the fact that a constantly augmented rate of absorption could be maintained by nervous stimulation lasting for about 30 min (Fig. 6) excludes the possibility that the observed effects were secondary to motility

The precise site of action of the intestinal sympathetic fibres on net water uptake is not known The distribution of the adrenergic fibres in the intestinal wall has been studied by several groups using Hillarp s fluorescent microscope technique (see Introduction) These studies have revealed a dense adrenergic innervation of neurones in the two nerve plexa of the intestinal wall In the mucosa the adrenergic fluorescence is concentrated to the crypt region while the villi to a large extent are devoid of any innervation Adrenergic fibres often accompany arterial and venous vessels although the stimulation of the sympathetic vasoconstrictor fibres does not decrease villous blood flow (Svanvik 1973)

The distribution of adrenergic fluorescence is compatible with several modes of action of the sympathetic nerves upon intestinal fluid transport although a direct nervous influence on the villus epithelium enhancing lumen to tissue water flux seems less likely due to the lack of adrenergic fibres in the villi This view is corroborated by preliminary experiments which suggest that the sympathetic influence on intestinal water transport is mainly caused by a decreased flux of water from tissue to lumen However at least three other modes of action seem possible First the sympathetic fibres may decrease an active secretion of solutes by the epithelial cells in the crypts

Second a decrease of crypt secretion may also occur secondary to a vasoconstriction in that region as proposed by Hultén et al (1977) The vascular effect may be mediated via a decrease of mean capillary hydrostatic pressure and/or via a marked decrease of nutritional supply to the epithelial cells A role for mean capillary hydrostatic pressure in intestinal water transport was suggested by the results reported in Figs 4 and 5 These show a more pronounced increase of net water uptake upon nervous stimulation when keeping femoral arterial blood pressure constant while an arterial pressure rise led to a reduction of the augmentation of water uptake Moreover Bohlen et al (1978) reported that pressure in the arterial vessel supplying the mucosa was decreased upon activation of the sympathetic vasoconstrictor fibres

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Effects of diethyl-stilboestrol on single fibres of frog skeletal muscle

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KHAN A. R. Effects of diethyl-stilboestrol on single fibres of frog skeletal muscle. Acta Physiol Scand 1979 106. 69–73 Received 8 Nov 1978. ISSN 0001-6772. Department of Pharmacology University of Lund, Sweden.

The effects of diethyl-stilboestrol (DES), one of the most potent estrogens, were studied on single muscle fibres of the frog. In relatively low concentrations (5 μM), DES greatly potentiates the twitch response of the fibre without significantly affecting the amplitude of the tetanus response. The twitch potentiation is accompanied by an increase in time to peak tension and marked prolongation of the relaxation phase. In tetanic response the half decay time after the last stimulus is also prolonged by DES. DES causes no changes in the resting or action potential of the muscle fibre. The S-shaped curve relating peak contracture tension and caffeine concentration is shifted towards lower caffeine concentrations by DES Dantrolene greatly suppresses the DES potentiated twitch. It is concluded that DES potentiates the twitch response and prolongs the relaxation time by inhibition of the calcium re-uptake by the sarcoplasmic reticulum.

Key words. Diethyl-stilboestrol, skeletal muscle, single muscle fibre, excitation-contraction coupling, contractile potentiation, action potential, membrane potential

Estrogens are known to influence the contractile activity of uterine muscle (Bengtsson 1973 Fuchs 1974 Batra & Bengtsson 1978) Their precise mechanism of action, however is not well understood. Previous studies suggest that diethyl-stilboestrol (DES), one of the most potent estrogens, influences the contractile activity of the uterus by inhibiting mitochondrial calcium uptake (Batra & Bengtsson 1972).

In skeletal muscle intracellular calcium concentration and thus the contractile activity of the muscle (for review see Sandow 1965) is regulated by a well organised sarcoplasmic reticulum (SR) It is therefore of interest to study the effects of DES on skeletal muscle. Experiments were performed on single muscle fibres. Evidence will be presented showing a great potentiation of the twitch response by DES Increase in twitch amplitude is associated with a marked prolongation of the relaxation phase suggesting an inhibitory action of DES on SR calcium uptake.

METHODS

Experiments were performed on single muscle fibres from the ventral head of the semitendinosus muscle of *Rana temporaria* Methods used for mounting, stimulation, temperature control and caffeine contractures were essentially similar to those described previously (Khan & Edman 1979).

Recording of membrane potential. The resting and action potentials were recorded, in the presence and absence of 5 μM DES using conventional glass capillary electrodes (Khan & Edman 1979)

In these experiments the bath temperature varied between 2.5 and 5.0°C from expt. to expt., but was maintained constant to within ±0.2°C throughout any particular expt., even during exchange of solutions.

Solutions. A stock solution (10 mM) of diethyl-stilboestrol (DES) was prepared in absolute alcohol from which 2, 5 and 10 μM solutions in Ringer were prepared. Maximum alcohol concentration was 1 ml/l of Ringer solution. A 9 μM solution of dantrolene sodium was prepared in Ringer solution.

All chemicals used were of analytical grade and dissolved in double distilled water Dantrolene sodium was obtained from Eaton Chemicals, Norwich, N Y U.S.A. and diethyl-stilboestrol was from Sigma Chemical Co St. Louis, U.S.A.

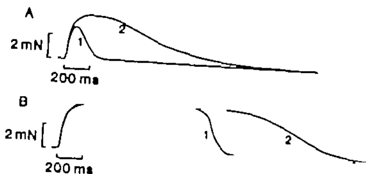


Fig 1 Effects of DES on isometric twitch (A) and tetanus tension (B) in frog single muscle fibre % Control normal Ringer solution In the presence of 5 μM DES. Stimulus marker below baseline

RESULTS

Twitch and tetanus response Effects of diethyl stilboestrol (DES) were studied on single muscle fibres in concentrations ranging between 2 and 10 μM In a concentration of 2 μM or higher DES greatly potentiated the twitch response without significantly affecting the amplitude of the tetanus (Fig. 1A and B) Maximum potentiation was obtained by 5 μM DES Twitch potentiation was inversely related to the twitch/tetanus ratio recorded in control Ringer solution

As illustrated in Fig 1A and B DES caused no change in the initial rate of rise of tension neither in twitch nor in tetanus response In the twitch response the increase in amplitude was associated with an increase in time to peak tension and a very pronounced prolongation of the relaxation phase (Fig. 1A) In tetanus response DES also caused a prolongation of the relaxation time after the last stimulus (Fig. 1B) The effects produced by DES were not reversible Repeated washing of the fibre with Ringer only lowered the amplitude of the potentiated twitch slightly

In concentrations 10 μM or higher of DES the fibre became irreversibly inexcitable to the electrical stimulus No contractures were induced by DES even when a 25 μM concentration was used

The effect of DES on caffeine induced contractures was also studied The peak tension developed before and after DES (5 μM) was plotted against log caffeine concentration As illustrated in Fig 2 DES shifted the S-shaped curve relating the contracture tension and caffeine concentration to the left

Dantrolene is known to have a powerful depressant effect on the isometric twitch In relatively low concentrations (10 μM) it greatly suppresses the twitch response of single muscle fibres (Hainaut & Desmedt 1974 Edman 1979) The effect of dantrolene on DES treated fibres was studied As illustrated in Fig. 3 dantrolene (9 μM) almost normalized the DES potentiated twitch

Membrane potential DES in a bath concentration of 5 μM which caused maximum twitch potentiation did not affect the resting membrane potential over at least 2 h as tested in 15 fibres of altogether 5 fibre bundles From Fig. 4 and Table it is evident that DES neither caused any significant change in the maximum rate of rise and decay nor in the overshoot of the action potential

DISCUSSION

In a muscle fibre inhibition of the calcium uptake by the sarcoplasmic reticulum (SR) would increase

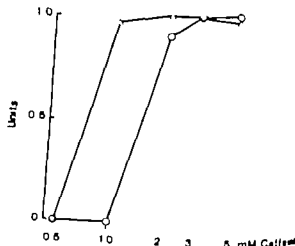


Fig 2 Effects of DES on peak contracture tension produced by different concentrations of caffeine O In the absence of DES. Δ In the presence of 5 μM DES Each point represents mean value of three different experiments

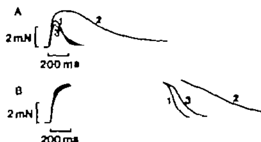


Fig. 3 Effects of dantrolene on twitch (A) and tetanus (B) responses of DES treated fibre. 1 Normal Ringer solution. 2. In the presence of 5 μM DES. 3 In the presence of DES and 9 μM dantrolene. Stimulus marker below baseline.

...e calcium concentration at the myofibrillar sites. If the inhibition by DES is large enough, it would result in an increase in the amplitude of the twitch response and a prolongation of the time to peak twitch tension. Furthermore, as calcium resequestration by SR governs muscle relaxation (Hasselbach & Makinose 1961, Ebashi & Lipmann 1962, Weber et al. 1963, Hasselbach 1964, Ebashi & Endo 1968), the twitch potentiation will be accompanied by a prolongation of the relaxation phase. The results presented here are consistent with the idea that DES potentiates the twitch response by inhibiting the SR calcium uptake according to the above mechanism. DES (100 μM) inhibits calcium uptake and calcium stimulated ATP-splitting in SR isolated from rabbit skeletal muscle (Khan, unpublished data) which also supports the above mentioned idea.

In mitochondria isolated from human myometrium, DES inhibits the calcium uptake (Batra & Bengtsson 1972). On the other hand, DES exerts an inhibitory action on the contractile activity of the intact uterine smooth muscle and it has been suggested that in this case *DES inhibits the influx of calcium* through the plasma membrane (Batra & Bengtsson 1978). This idea is consistent with the findings that the contractile activity of the uterine smooth muscle to a large extent, is dependent on the extra-cellular calcium (Edman & Schild 1962, Bengtsson 1978). It is possible that DES exerts a similar inhibitory action on the plasma membrane of the skeletal muscle but it is unlikely to produce a noticeable effect, since in this case extra-cellular calcium plays little role in contractile activity (Edman & Grieve 1964, Andersson & Edman 1974, Armstrong et al. 1972 (for review see Fuchs 1974).

DES does not cause any changes in the resting and action potentials of the fibre. These results suggest that twitch potentiation caused by DES is not due to a membrane effect. DES is a very lipid soluble substance and like other estrogens (Jensen et al. 1969, Evans & Hahnel 1971) may readily penetrate the cell membrane. It is therefore possible that the drug directly affects intracellular structures like the sarcoplasmic reticulum. The membranes of SR have high amounts of phospholipids. Furthermore the SR lipid phase contains large amounts of lecithin (60–73%) (Balzer & Khan 1975, Tada et al. 1978) which have a high percentage of 18-carbon unsaturated fatty acids (Balzer & Khan 1975). Previous studies suggest that drugs like chlorpromazine, reserpine and prenylamine are bound to unsaturated fatty acids of the SR lipid phase and thereby exert their inhibitory action on the SR calcium uptake (Balzer et al. 1968). A similar binding

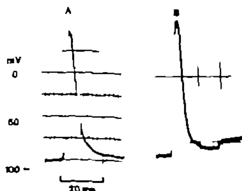


Fig. 4 Effects of DES on intracellularly recorded action potential of frog single muscle fibres. A. Normal Ringer solution. B. In the presence of 5 μM DES. The records in A and B are from different fibres.

Table I *Effects on resting and action membrane potentials of 5 μM DES added to normal Rin (Mean ± S E)*

Frog muscle fibre bundles exposed to DES for 15–20 min before recording the resting and action potentials. No. of fibres for each measurement given within brackets

	Resting potential (mV)	Action potential			
		Overshoot (mV)	Maximum rate of rise (V/s)	Maximum rate of decay (V/s)	Duration at −25 mV level (ms)
Control	88 3±2 4 (11)	42 9±2 9 (11)	6[illegible].4±5 1 (11)	31.[illegible]±1 6 (11)	5 2±0.3 (11)
DES (5 μM)	87.3±2 9 (15)	41 9±4 0 (15)	63 6±4 0 (15)	29.6±2.4 (15)	5 0±0.2 (15)

of DES to the lipid phase of SR might be responsible for the observed twitch potentiation and prolongation of the relaxation phase

Previously it has been considered that the effects produced by caffeine on excitation-contraction of muscle are due to inhibition of the calcium pump of the SR (Weber 1968) Recent studies of Endo (1975) on the other hand suggest that caffeine potentiates the twitch and in higher concentration induces contractures by enhancing calcium release from the SR If DES inhibits the calcium re-uptake by the SR then calcium released by caffeine would accumulate and induce a contracture by lower than normal concentrations of caffeine The results showing that DES shifted the S-shaped curve relating peak tension and caffeine concentration towards lower drug concentrations are consistent with that idea Dantrolene a powerful muscle relaxant has been suggested to inhibit depolarization induced calcium release (Hainaut & Desmedt 1974 Desmedt & Hainaut 1977) Inhibition by DES of SR calcium re sequestration would tend to increase calcium concentration at myofibrillar sites As this effect is dependent on the first step i e the calcium release the twitch potentiating effect of DES would be much reduced by dantrolene as was observed in the present experiments

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ɪnin aldosterone and cortisol during ethanol toxication and hangover

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LINKOLA, J FYHRQUIST F & YLIKAHRI, R.. Renin, aldosterone and cortisol during ethanol intoxication and hangover Acta Physiol Scand 1979 106:75–82. Received 9 Nov 1978. ISSN 0001-6772. Minerva Institute for Medical Research, Helsinki and Third Department of Medicine, University of Helsinki, Finland.

The effect of ethanol intoxication and hangover on plasma renin activity (PRA), plasma aldosterone (PA) and plasma cortisol (PC) concentrations was studied in 7 healthy supine men in controlled clinical conditions during 18 h beginning at 6 p.m. Large individual variation was observed in the response of PRA, PA and PC to ethanol. Following ethanol, stimulation of PRA was observed at the 14th and the 16th hour ($P<0.05$), of PA at the 4th and the 6th hour ($P<0.01$ and $P<0.05$ respectively) and of PC at the 4th and the 14th hour ($P<0.01$ and $P<0.05$ respectively) Ethanol ingestion suppressed PC during the first hour ($P<0.02$). Water ingestion at 8 a.m. suppressed PA between the 14th and the 16th hour (8–10 a.m) in control and ethanol experiment ($P<0.01$ and $P<0.005$ respectively). There was a dissociation between PRA and PA, but intra-individually PRA and PA correlated fairly or well. Plasma arginine vasopressin (AVP) and PC were also significantly correlated. The results suggest that changes in PA and PC as well as the dissociation of PRA and PA after ethanol ingestion might be partly related to dehydration and to the increased secretion of hypothalamic and pituitary hormones as well as to sodium and potassium balance. There was biphasic effect of ethanol, including an initial suppression of PC and subsequent increase of PC, PRA and PA. Upright posture appears to exaggerate the stimulating affect of ethanol on PRA PA and PC.

Key ords Renin, aldosterone, cortisol, ethanol

oderate doses of ethanol appear to increase plasa renin activity (PRA) and plasma aldosterone A), plasma cortisol (PC) (Jenkins & Connolly 68, Farmer & Fabre 1975 Ylikahri et al. 1978) nd plasma arginine vasopressin (AVP) (Linkola et l 1978) concentration in man. Preliminary obser ations ha e shown an initial suppression of PA uring ethanol intoxication (Linkola et al. 1976) Moreover it has been traditionally emphasized that thanol inhibits vasopressin release (Wallgren & arry 1970) These data may suggest a biphasic ffect of ethanol on water and electrolyte etabolism, an initial suppression of hypothalamic nd adrenocortical function followed by a phase of timulation.

The preliminary study (Linkola et al. 1976) alady revealed high PRA and PA values during angover Similar data is now a ailable on PC Ylikahri et al. 1978). Although a significant correlation was previously observed between PRA and PA values (Linkola et al. 1976), the possibility remained that hypothalamic functions, ACTH and changed sodium-potassium balance may also affect PA and cause dissociation of PRA and PA during ethanol intoxication and hangover Dehydration after ethanol diuresis is thought to be partly responsible for increased PRA (Linkola et al 1976) and AVP (Linkola et al 1978) values. Furthermore it has been suggested that AVP potentiates ACTH release (Yates et al. 1971) On the other hand dehydration may be a reason for a discrepancy between PRA and PA at least in a patient with diabetes insipidus and adipsia (Schalekamp et al. 1975) Dissociation of PRA and PA has been also reported in the syndrome of inappropriate antidiuretic hormone secretion (SIADH) (Fichman et al. 1974) during changes in sodium balance (Armbruster et al. 1975 Michelakis & Horton 1970) in primary aldos-

teronism (Vetter et al 1974 Kem et al 1973) and during hypoglycemia (Hata et al 1976) Because ethanol strongly changes the steady-state of the body by causing hypoglycemia emesis (Wallgren & Barry 1970) primary suppression of serum [K] increased serum [Na] (Linkola et al 1976) and sometimes inappropriately high plasma AVP values (Linkola et al 1978) it appeared of interest to investigate in detail changes in PRA PA and PC and their interrelationship with each other and with AVP (results published recently by Linkola et al 1978) during ethanol intoxication and hangover Numerous reports have focused on a possible role for ethanol in hypertension (Ramsay 1977*a* Beevers 1977 Klatsky et al 1977 Mathews 1976 Ramsay 1977*b*) in hypercortisolism (Smals & Koppenborg 1977) and in hyponatremia of beer drinkers (Hilden & Svendsen 1975 Phillips & Pajn 1975) Because these disturbances may be related to PRA antiotensin II (A II) AVP PC and PA they are also briefly discussed

MATERIALS AND METHODS

Seven healthy men, aged 25–35 volunteered for the experiments carried out in a metabolic ward. For 5 days the subjects received isocaloric diet (about 30 kcal day kg) containing 100 mmol sodium and 90 mmol potassium per day In the morning of the fifth day they received 2/3 the calories of that day and all sodium as powdered NaCl. Then beginning at noon they were supine for 22 h and fasted to 6 p.m From 6 p m. to 9 p m. they received deionized water 10 ml/kg. Next morning, at 8 a.m they received water 5 ml/kg. At 10 a.m they assumed a standing position walking slowly around They had nothing to eat until noon. After that they had a meal (about 30 kcal/kg 100 mmol sodium and 90 mmol potassium)

Beginning in the morning of the seventh day the same procedure as two days earlier was repeated except that instead of water in the evening the subjects now received 15% w/v ethanol 10 ml/kg Blood samples were taken by venipuncture in control and ethanol experiments hourly in the evening from 6 to 10 p.m then at 12 p m 6, 8 and 10 a.m. and at noon Urine was collected 6–9 p m 9–12 p m 0–6 a.m. and 6–12 a.m

PRA was determined by radioimmunoassay (Fyhrquist et al 1976) PA as described previously (Linkola et al 1976) PC by protein binding assay (Murphy 1967) and [Na] and [K] of urine were measured as described (Ylikahri et al 1974) Hewlett Packard 9810 A calculator was employed in paired *t*-tests and linear regression analyses.

RESULTS

Renin. Individual changes in PRA during control and ethanol experiments are presented in Figs 1–4 and the mean values in Fig. 4 Although PRA values during ethanol intoxication exceeded controls in subjects (all but II and VII) at 10 p m. and in subjects (all but I) at 6 a m. the difference between ethanol and control experiment was not significant until 8 a.m and 10 a m ($P<0.05$) PRA levels were higher in the morning than in the evening in both control and ethanol experiment In control and ethanol experiment PRA responded significantly to upright posture ($P<0.05$) During hangover the response of PRA to upright posture was exaggerated in 4 subjects (I II III and VII) Subjects having nausea and vomiting (II III and IV) had the highest differences in PRA between control and ethanol experiment These subjects were the only ones experiencing subjective hangover symptoms (Ylikahri et al 1974) Nausea and vomiting were not clearly temporally related to any measured parameters

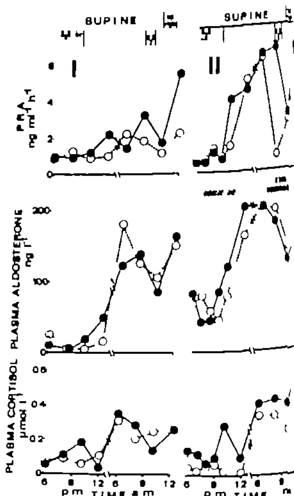
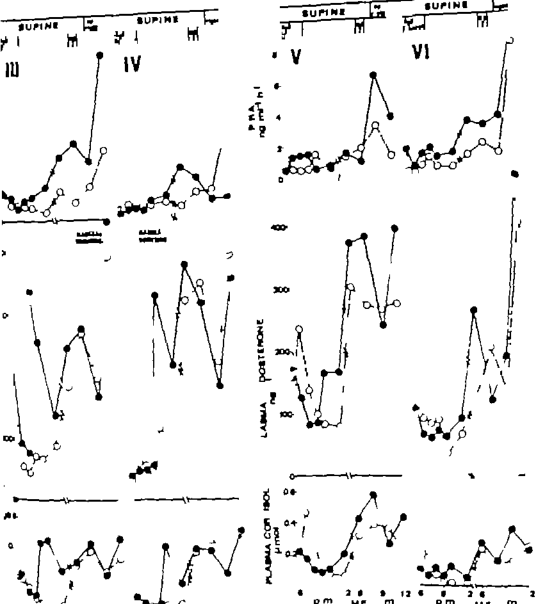


Fig 1 Plasma renin activity (PRA) aldosterone (PA) cortisol (PC) in subjects I and II in control (O—O) ethanol (●—●) experiment.



2 Plasma renin activity (PRA) aldosterone (PA) and ...ol (PC) in subjects III and IV in control (O—O) ... ethanol (●—●) experiment.

Fig 3 Plasma renin activity (PRA) aldosterone (PA) and cortisol (PC) in subjects V and VI in control (O—O) and ethanol (●—●) experiment.

...ldosterone During the first 2 h of intoxication ... values were below the controls in 6 subjects (all ... III) (Figs. 1–4) Also in subject III PA displayed ...more pronounced decrease after ethanol than af... water during this time This initial suppression ...s, however not statistically significant. Subjects ...and IV having also nausea and vomiting, had high PA peaks during ethanol intoxication. In the control experiment PA and PC correlated at 6 p.m. (r=0.771 $P<0.05$), at 7 p.m. (r=0.930, $P<0.01$) at 8 p.m. (r=0.763 $P<0.05$) at 8 a.m. (r=0.736 $P<0.05$) and at 10 a.m. (r=0.882, $P<0.01$) (Table 1). In ethanol experiment PA and PC correlated at 6 p.m. (r=0.958 $P<0.001$) at 9 p.m. (r=0.995

teronism (Vetter et al 1974 Kem et al 1973) and during hypoglycemia (Hata et al 1976) Because ethanol strongly changes the steady state of the body by causing hypoglycemia emesis (Wallgren & Barry 1970) primary suppression of serum [K] increased serum [Na] (Linkola et al 1976) and sometimes inappropriately high plasma AVP values (Linkola et al 1978) it appeared of interest to investigate in detail changes in PRA PA and PC and their interrelationship with each other and with AVP (results published recently by Linkola et al 1978) during ethanol intoxication and hangover Numerous reports have focused on a possible role for ethanol in hypertension (Ramsay 1977*a* Beevers 1977 Klatsky et al 1977 Mathews 1976 Ramsay 1977*b*) in hypercortisolism (Smals & Koppenborg 1977) and in hyponatremia of beer drinkers (Hilden & Svendsen 1975 Phillips & Pain 1975) Because these disturbances may be related to PRA angiotensin II (A II) AVP PC and PA they are also briefly discussed

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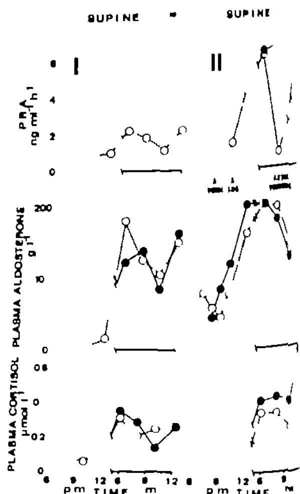


Fig 1 Plasma renin activity (PRA) aldosterone (PA) cortisol (PC) in subjects I and II in control (O——O) ethanol (●—— ●) experiment

ble 1 *Intra-individual correlations between AVP and PC PC and PA and PRA and PA in subjects I–VII ing control (C) and ethanol (E) experiment*
correlation coefficient using linear regression analysis, *P*=probability NS=not significant

AVP–PC				PC–PA				PRA–PA			
C		E		C		E		C		E	
	P<	*r*	*P*<		*P*<		*P*<		*P*<		*P*<
0.322	NS	0.746	0.05	0.969	0.001	0.779	0.05	0.871	0.01	0.762	0.05
0.744	0.05	0.854	0.01	0.802	0.01	0.527	NS	0.765	NS	0.873	0.001
0.787	0.01	0.768	0.01	0.857	0.01	0.884	0.001	0.817	0.01	0.708	0.05
0.730	0.05	0.508	NS	0.966	0.001	0.957	0.001	0.658	0.05	0.776	0.01
0.593	NS	0.941	0.001	0.807	0.01	0.947	0.001	0.476	NS	0.284	NS
0.238	NS	0.299	NS	0.518	NS	0.635	0.05	0.956	0.001	0.964	0.001
0.735	0.05	0.875	0.001	0.895	0.001	0.766	0.01	0.907	0.001	0.871	0.01

…d or equalled the control values in all but one …ject (VI). Individual differences were large, and …n the difference was not statistically significant

…SCUSSION

…e results are essentially consistent with earlier …a, supporting a dominantly stimulatory effect of …anol on PRA, PA and PC (Farmer & Fabre 1975 …kins & Connolly 1968 Linkola et al. 1976, …habri et al. 1978) when studied on an 18-hour …iod basis. The results also emphasize the large …ividual variation and the importance of differen…ting between early and late endocrine responses …er ethanol ingestion. While, initially a suppres…n of PC was observed, stimulated release of re… aldosterone and cortisol followed during later …ases of ethanol intoxication and particularly dur… hangover.

PRA did not increase significantly during ethanol …oxication (Fig. 4) as in an ambulatory study (Lin…la et al. 1976). Thus, posture appears to modulate …e extent to which PRA is changing after ethanol ingestion. There may be also individual differences in response time of PRA to ethanol. It has been previously suggested that stimulation of renin-aldosterone axis during ethanol intoxication and hangover is due to dehydration and increased activity of the sympathetic nervous system (Linkola et al. 1976). The present work describes in more detail the relationships between renin, aldosterone and cortisol and their correlation with vasopressin, but does not afford additional data on the role of various physiological changes in the stimulation of PRA after ethanol ingestion.

Earlier data (Linkola et al. 1976) showed a decrease of PA during the early phase of ethanol intoxication. Suppression of PA caused by ethanol ingestion was also seen in these experiments in 6 subjects (all but I) but the great variability of individual PA levels probably blunted the significance of the initial PA suppression. The results indicate that PA is not governed only by PRA. Initial decrease of PA was previously thought to be related to suppressed PRA Linkola et al. 1976). However there were no statistically significant initial decre-

…ble 2 *Urinary sodium (N) and potassium (K) excretion of subjects I–VII in normal and the corresponding N /K ratios …ing 4 periods in control (C) and ethanol (E) experiment*
…ean and S D

	6–9 p.m.		9–12 p.m.		0–6 a.m.		6–12 a.m.		Total	
	C	E	C	E	C	E	C	E	C	E
	44±7	25±12	25±7	13±7	7±5	7±4	17±9	17±12	84±12	59±14
	14±4	9±2	6±2	3±3	5±1	9±5	20±5	27±13	40±5	69±62
	4.1±1.7	2.8±1.9	4.7±2.3	4.9±3.6	1.2±0.7	0.9±0.7	0.9±0.5	0.8±0.6	2.1±0.4	1.2±0.6

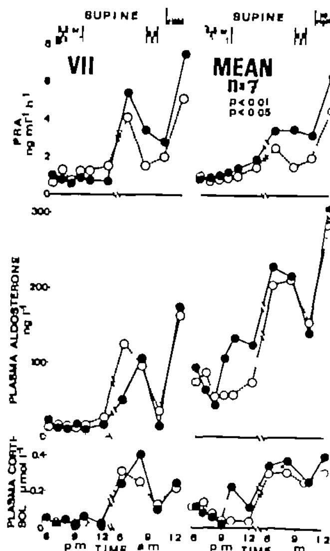


Fig 4 Plasma renin activity (PRA) aldosterone (PA) and cortisol (PC) in subject VII and the mean values ($n=7$) in control (O——O) and ethanol (●- - -●) experiment

$P<0\ 001$) and at 10 p m ($r=0\ 807$ $P<0\ 05$) During hangover PA values exceeded or equalled controls in 5 subjects (all but I and VII) at 6 a m but there was a decrease below controls in all subjects during the morning followed by an increase above con trols in 5 subjects (all but II and IV) There was a significant decrease in PA after water ingestion between 8 and 10 a.m in both control and ethanol experiment ($P<0\ 01$ and $P<0\ 005$ respectively) Subjects II and IV having emesis during ethanol experiment also had lower PA values than controls at noon during hangover In control and ethanol experiment the increase of PA after upright posture (10–12 a.m) was significant ($P<0\ 01$ and $P<0\ 005$ respectively) During hangover the response of PA to upright posture was exaggerated in 5 subjects (all but II and IV) The difference between ethanol and control experiment was significant at 10 p ($P<0\ 05$) and at 12 p m ($P<0.01$). PRA did correlate with PA neither in control nor in expt, when compared chronologically group but fairly to well when intra-individually (Table 1) PA levels were higher in the morning than in the evening in both control and ethanol expts

Cortisol There was an initial decrease of PC after water in 4 subjects (II III IV and VI) and after ethanol in 6 subjects (all but I) (Figs. 1-4) ethanol experiment this initial decrease between and 7 p m. was significant ($P<0\ 02$). At 10 p.m was below starting value in 6 subjects (all but VI after water ingestion but above that after ethanol 5 subjects (all but V and VII) The difference in values between control and ethanol expt was significant in the evening only at 10 p m. ($P<0$ During hangover PC exceeded controls in 6 subje (all but VII) at 6 a.m Water ingestion at 8 a.m h no significant effect on PC although at 8 a.m t difference between ethanol and control expt significant ($P<0\ 05$) but not at 10 a.m. or at 10 During ethanol expt the increase of PC after right posture was significant ($P<0\ 025$), but n during the control one Thus the response of PC upright posture was exaggerated

Plasma AVP results urine outputs and H ethanol concentrations were given previously (Li kola et al 1978) During control expt PC and A correlated at 10 p m ($r=0\ 762$, $P<0\ 05$) and du ethanol expt at 6 p m ($r=0\ 803$ $P<0\ 05$) at 9 p ($r=0\ 985$ $P<0\ 001$) and at 10 p m (r 0 $P<0\ 05$) Intra individually PC and AVP corre during control expt in 4 subjects (II III IV VII) and during ethanol expt in 5 subjects (al IV and VI) (Table 1) PC levels were higher in morning than in the evening in both control ethanol expts

Urine sodium and potassium During ethanol toxication (6–12 p m) 6 subjects (all but I) excr less sodium than during the corresponding co time (Table 2) 6–9 p m $P<0\ 005$ and 9–12 p $P<0\ 01$ Potassium output was also smaller ethanol expt than in control expt between p m ($P<0\ 05$) During hangover (0–12 a.m) 6 jects (all but VI) excreted more potassium during the control time but individual diffe were large Totally sodium excretion was lo during ethanol expts. than during control expt all subjects ($P<0\ 005$) Potassium excretion higher during ethanol expt than during co

ration 6–9 p m. in spite of high PA values. This ect may be centrally mediated (Andersson 1977) cause he had simultaneously the highest AVP ues (Linkola et al. 1978). Prostaglandins may o account for this effect (Zins 1975) although cific evidence is lacking.

Ethanol drinking may be associated with hyper nsion (Ramsay 1977*a* Beevers 1977 Klatsky et 1977 Mathews 1976, Ramsay 1977*b*). In this ntext, the role of h er dysfunction and cortico- roid excess have been widely discussed, but PRA d A II ha e not been considered These factors ay however play a significant role in high blood essure after acute and chronic ethanol drinking. e mechanism of corticoidism among alcohol nkers (Smals & Koppenborg 1977) is unknown. e correlation observed between AVP and PC ay indicate an association between AVP and CTH and thus may suggest a role for AVP-stimu- ed ACTH release in adrenocortical hyperfunc n among alcohol abusers.

Cortisol release was stimulated during hangover d exaggerated when upright posture was as- med. Moreover the stimulation of renin-angio- nsin system would even contribute to the adren- gic activation (Peach 1977) after ethanol drink g, at least during hangover This study pro ides idence that the non-specific stress response to hanol (Wallgren & Barry 1970) may be partly ediated through the actions of ethanol on water nd electrolyte metabolism. Accordingly mainten- nce of a positive hydration state during ethanol toxication might counteract the stress-hormone lease

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ment of PRA in the present study even though rapid fluctuations in PRA during the first hour can not be excluded The similarity of initial PA and PC patterns in 4 subjects (III V VI and VII) (Figs 2 3 and 4) suggests that PA may also reflect ACTH release Significant correlations (Table 1) between PA and PC provide additional evidence for such a suggestion The results of Farmer & Fabre (1975) and partly those of ours showing a more rapid initial decrease of PA than PC or PRA may point to other alternative mechanisms controlling aldosterone release Ethanol may directly suppress aldosterone as well as cortisol biosynthesis and release although we are aware of no data to support this assumption Ethanol induced changes in sodium-potassium balance (Wallgren & Barry 1970 Linkola et al 1976) may affect aldosterone biosynthesis too Thus lowered serum [K] during ethanol intoxication may be partly responsible for the decrease in PA Increased serum [Na] may be another contributing factor

Substantial amounts of potassium may be lost by vomiting. Because this occurs primarily at the debt of intracellular stores adrenocortical cells too may loose potassium Aldosterone biosynthesis may have been suppressed by this mechanism in subjects II and IV during hangover resulting in dissociation of PRA and PA In subject II PA values were poorly understandable on the basis of PC (ACTH) Low PA values in subject VII may have been associated with his high urinary potassium output during ethanol expt (Table 2) This subject however had a remarkably low response in all measured hormone and enzyme variables during the expts (see also Linkola et al 1978) He also had the highest serum [K] in the beginning of the expt (4 3 mmol/l) while subjects III and IV having high responses had the lowest serum [K] (3 8 mmol/l) (Linkola et al 1978) The significance of K status and serum [K] in this context remains to be studied

PA and PC peaks were simultaneous in subjects III and IV during ethanol intoxication (Fig 2) These peaks also coincided with AVP peaks observed during the same expt (Linkola et al 1978) High AVP levels in the subjects experiencing nausea and vomitus (II III and IV) suggested that such toxic effects of ethanol may be associated with the hydration state of the body and with the sensitivity of AVP triggering mechanisms (Linkola et al 1978) AVP has been thought to release ACTH directly or by potentiating the actions of corticotropin releasing factor (CRF) (Yates et al 1971) Our results support this hypothesis indicating a correlation between AVP and PC The correlation between PC and PA may indicate a connection between ACTH and PA Because the nature of CRF is unknown and other affectors, such as neurotransmitters (Smythe 1977) and prostaglandins (Hedge 1977) were not measured their role in ACTH release remains hypothetical Jenkins & Connolly (1968) suggested that ethanol stimulates cortisol secretion by stimulating ACTH release though neither they nor Suzuki et al (1972) suggest an association between AVP and ACTH very likely because ethanol is traditionally thought to cause AVP suppression only

In an ambulatory study (Linkola et al. 1976) PRA and PA were significantly correlated. However emesis did not occur in that investigation. In the present study individual PRA and PA values correlated fairly to well (Table 1) Interestingly in subject (V) with poor PRA–PA correlation, a significant PC–PA correlation was observed. Conversely in subject VI with an absent AVP–PC correlation and a weak PC–PA correlation, there was an excellent PRA–PA correlation. One possible reason for absent correlation between PRA and PA when determined in samples collected simultaneously in all subjects was the time lag between renin release and the resulting production of aldosterone Differences in PRA–PA time lag may explain why intra individual correlations (Table 1) were less pronounced than those of chronologically obtained groupwise data It should be pointed out that correlation values cannot prove the functional interrelationship

Higher mean PA values during the ethanol expt than during the control one may account for the sodium retention and potassium output occurring during the influence of ethanol (Table 2) On the other hand Pierson et al (1977) have consistently reported that ethanol decreases intracellular potassium while increaseng intracellular sodium The urinary excretion of electrolytes may reflect changes at the intracellular level Beer drinking has been causally correlated to hyponatremia (Hilden & Svendsen 1975 Phillips & Palm 1975) Apparently when ethanol is ingested with excessive amounts of fluid or in prehydrated state natriuresis occurs instead of sodium retention (Linkola 1975) In subject III sodium excretion was the highest after

ɜ effect of centrally administered TRH on blood pressure ɜrt rate and ventilation in rat

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KOIVUSALO F PAAKKARI L LEPPÄLUOTO, J & KARPPANEN H. The effect of centrally administered TRH on blood pressure, heart rate and ventilation in rat. Acta Physiol Scand 1979 106: 83–86. Received 3 Dec. 1978. ISSN 0001-6772. Depts. of Physiology and Pharmacology University of Oulu, Finland.

Different doses (3 16, 80 400 and 2 000 ng per rat) of TRH or alternatively saline were infused into the lateral ventricle of urethane-anaesthetized rats and blood pressure, heart rate respiration rate and tidal volume were recorded. A dose of 3 ng TRH caused a significant elevation of blood pressure and heart rate whereas 16 ng was needed for respiration rate to be elevated. There was no change in respiratory minute volume during the experiment. We conclude that TRH has profound physiological effects and that TRH given centrally is a potent hypertensive, chronotropic and tachypnoeic agent.

Key words TRH blood pressure heart rate, ventilation, rat

he tripeptide thyrotropin-releasing hormone TRH) was isolated from the hypothalamus on the asis of its ability to release thyrotropin from the ituitary gland (Burgus et al 1969 Nair et al 1970) Although the hypothalamus has the highest TRH oncentration, the hormone has been found hroughout the central nervous system of several pecies (Jackson et al 1974 Winokur et al 1974 ardon et al 1977 Leppäluoto et al 1978). The ide distribution suggests other functions of TRH han thyrotropin r lease and there is now evidence of a profound effect of TRH on the nervous ystem TRH antagonizes the sedation of ethanol and barbiturates and other depressant drugs (Breese et al. 974, Breese et al 1975), and potentiates DOPA induced excitation (Plotnikoff et al. 1972). When injected intr entricularly into rabbits, TRH induces achypnea, hyperthermia and behavioural excitation and this effect of TRH on beha iour is blocked by morphine (Horita et al. 1976).

Recently attention has been directed to the physiological effects of TRH Intracisternal injection of TRH in rabbit is reported to produce EEG activation and rise in blood pressure (Beale et al. 1977) The analeptic effect of TRH has been observed in mouse rat and rabbit using several differ ent routes of administration. A rise in blood pressure is therefore to be expected with intracerebroventricular administration of TRH in rat. The present study shows the effect of intracerebroventricularly administered TRH on mean blood pressure heart rate and ventilation in the rat.

MATERIAL AND METHODS

Male Sprague-Dawley rats (250–300 g) were anaesthetized with urethane (1.5 g/kg v). The trachea was cannulated with polyethylene tube and the rats were allowed to breathe spontaneously Ventilation was measured with hot ire air flowmeter placed close to the opening of the tracheal tube (details will be published). The signal from the flowmeter ere first stored on tape (Store 7 Instrumentation recorder Racal Thermionic) and later integrated with an integrator (Hewlett Packard 3380 A) to obtain tidal volume given as arbitrary units of the machine Mean blood pressure and heart rate were measured directly from the left femoral artery by means of pressure transducer (1280 C), pressure amplifier (8805 C) and rate computer (8812 A) made by Hewlett Packard. The rats were mounted to stereotaxic instrument and an injection needle was introduced into the lateral cerebral ventricle A polyethylene catheter filled with the solution to be infused, was attached to the needle The volume infused was 10 µl and it was allowed to flow by virtue of hydrostatic pressure (45 cmH_2O). The infusion lasted 10–15 s. TRH (Ferring Ltd, Sweden) was dissolved in 0.9% NaCl and infused in one dose (400 ng per rat) or in cumulative doses (3 16, 80, 400 and 2 000 ng per rat). The interval be-

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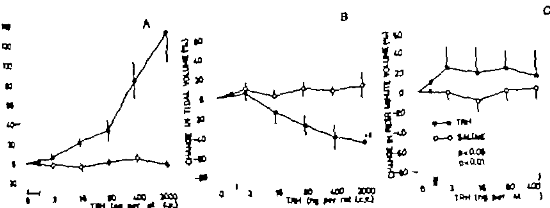
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ig 3 The effect of increasing doses of i.c. infused TRH or saline on respiration rate (panel A), tidal volume (panel B) and respiratory minute volume (panel C). Each symbol is given as mean ± S.E. (=4-6) and represents the values obtained 10 min after infusion. Asterisks indicate a statistical significance between TRH and the corresponding saline infusions

ere relatively long lasting, the dose response curves of TRH were based on cumulative dosages.

Before infusion, the m.b.p. was 96±4 in the test group and 97±5 mmHg in the control group. Repeated i.c. infusions of TRH resulted in a dose-related increase in m.b.p. in a semilog plot, whereas no rise occurred in the control rats (Fig. , panel A). A dose of as little as 3 ng TRH resulted in a significant rise in m.b.p. compared to the control group (15± mmHg, $p<0.001$).

When measuring h.r. the starting value for the test group was 381±21 and for the control group 10±15 beats/min. The difference between the groups is not significant ($p>0.2$). Administration of TRH induced a dose related rise in h.r. and the 3 ng dose, already resulted in a significant rise compared to the control group (40±11 beats/min, <0.01) (Fig. 2 panel B). After 80 ng per rat i.c.v. a plateau of about 460 beats/min was reached.

The r.r. was 119±3 and 1 4±9 r/min for the test group and control group respectively before starting the infusions. The r.r. did not rise with 3 ng of TRH but a significant rise (20±4 r/min $p<0.05$) was observed after 16 ng compared to the control (Fig. 3 panel A). The control level did not change during the experiment. The increased r.r. paralleled a simultaneous decrease in tidal volume and as a consequence there was no significant change in respiratory minute volume (Fig. 3 panels B and C).

The cornea reflex was tested after 3 ng TRH. This reflex became positive in all anesthetized rats given TRH but remained negative in the control rats (Table 1).

DISCUSSION

Our results confirm the previously reported pressor effect of centrally administered TRH. Delbarre et al. (1977) injected TRH into the lateral ventricle of chloralosed cats and Beale et al. (1977) into the cisterna magna of curarized rabbits and both groups observed a rise in blood pressure. However Beale et al. did not find any rise in heart rate, whereas our results and those of Delbarre et al. do. The different species, anesthetics or the different route of infusion may explain these varying results.

The fact that a dose as small as 3 ng infused into the lateral ventricle of the rat caused many cardiovascular and respiratory changes indicates that we are dealing with a substance which works at a profound physiological level. Our experiments demonstrate that TRH is one of the most potent centrally effective hypertensive agents. It is possible that TRH has different effects on cardiovascular and respiratory centers because m.b.p. and h.r. increased at a dose of 3 ng, but r.r. not before 16 ng. The different sensitivity to TRH could also be explained by varying penetration of the drug from

Table 1 *Effect f v infused TRH or saline on cornea reflex*

	No. of rats	Positive cornea reflex	Negative cornea reflex
Saline	6	0	6
3 ng TRH	7	7	0

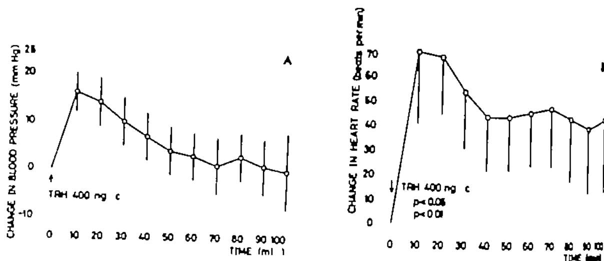


Fig 1 The effect of a single dose of 400 ng of i.c v infused TRH on blood pressure (panel A) and heart rate (panel B) Each symbol is given as mean ±S E. ($n=5$) Asterisks indicate a statistical significance between the preinfusion value and the values measured thereafter calculated with Student s t-test for paired comparison.

tween the doses was 20 min Control rats were infused with 0.9% NaCl in the same manner The registrations were continued during the infusion. At the end of each experiment methylene blue was injected and the proper position of the needle in the cerebral ventricle was ascertained in the autopsy The results given as means ± S E and the effect of TRH and saline tested by using Student s t-test for independent observations or t test for paired observations when indicated

RESULTS

Intracerebroventricular (i c v) administration of TRH in rat gives a rise in mean blood pressure (m b p) respiration rate (r r) and heart rate (h r)

The maximal hypertensive effect after a single dose of 400 ng per rat of TRH was reached within min and the pressure fell to the preinjection level (99±4 mmHg) in about an hour (Fig. 1 panel A) The onset of the rise occurred within ? min of the infusion being completed The peak of the corresponding tachycardia was also reached within 10 min and the heart rate remained about 45 beats/min higher as compared to the preinjection level (362±48 beats/min) but this difference was not statistically significant (Fig 1 panel B) Since the cardiovascular effects of i c v administered TRH

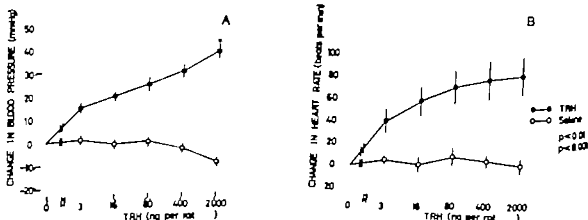


Fig 2 The effect of increasing doses of i.c v infused TRH or saline on blood pressure (panel A) and heart rate (panel B) Each symbol is given as mean ± S E. ($n=8$) and represents the values obtained 10 min after infusion Asterisks indicate a statistical significance between TRH and the corresponding saline infusions

ta Physiol Scand 1979 106, 87–89

eta-adrenergic inhibitory interference with myogenic ascular reactivity during experimental intervention

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e concept of myogenic vascular control has been uch debated since it was originally proposed by y liss in 1902. Some apparent problems have aris- in the search for conclusive evidence. For in- ance, it is difficult to design specific myogenic test periments which do not simultaneously lead to terference with the local metabolic vascular con- ol system. Another puzzling feature is that even emingly well designed experiments can exhibit oor or occasionally failing, myogenic reactivity .g. Baez, Laidlaw & Orkin 1974 Johnson & Inta- etta 1976, Grände, Lundvall & Mellander 1977) ch experience must not necessarily be taken as idence against the myogenic theory but might erely imply that the myogenic control system, by me mechanism, is vulnerable to experimental in- rvention. It is thus a common empirical observa- on that myogenic reactivity can be severely de- essed under experimental conditions involving auma, for instance such related to anesthesia, rgery accidental hemorrhage or hypothermia, en slight stress to the experimental animal can pair myogenic reactivity significantly (e.g. Lund- all, Mellander & Sparks 1967 Grände et al. 1977) a therefore reasoned that possible stress-induced lease of catecholamines from the adrenal medulla, a β-adrenergic effect, might be a factor con- ributing to decreased myogenic reactivity during xperimental intervention. This hypothesis seemed o be confirmed by the present experiments.

METHODS

he study was performed on 8 cats. Observations were ade on the acutely sympathectomized lower leg muscles f the hindlimb with intact circulation (for details of ethodology see Grände et al. 1977). The muscle region as enclosed hermetically in a plethysmograph so that an crease in vascular transmural pressure (P_T) could be pplied at a constant rate (dP_T/dt) by lowering plethys- ograph pressure. By observations of arterial blood flow nd of arterial pressure, small artery pressure, small vein ressure, and venous outflow pressure and with the use of differential pressure transducers and electronic divider circuits (Grände & Borgström 1978) continuous recordings of the resistance responses evoked in the whole vascular bed in 'proximal' arterial vessels, in the 'microvessels' (diam. 20–25 μm and smaller) and in large veins could be obtained both during the dynamic phase of the transmural pressure increase (dynamic myogenic response) and in the steady state phase of constant increased pressure (static myogenic response). Since the recently described dynamic response is a very pronounced myogenic reaction compared to the static response and since the former is confined to the microvessels (Grände et al. 1977), emphasis in the present study will be placed on 'microvascular' myogenic reactivity

RESULTS

Myogenic reactivity to a standardized transmural pressure stimulus in terms of a P_T increase of 30 mmHg applied at the rate (dP_T/dt) of 2.5 mmHg/s was tested on the muscle vascular bed in all expts. just after completion of surgery In 4 of the cats such tests were repeated during a following period of rest lasting for more than 1 h. In the other 4 cats an effective β-adrenergic blockade (propranolol 1 mg/kg tissue) was instituted immediately after the first test of myogenic reactivity upon which re newed tests were undertaken. The average results (mean values ± S.E.) are presented in Fig. 1 where the left histogram refers to the amplitude of the peak microvascular dynamic myogenic constrictor response and the right one to data for basal microvascular tone. The white bars refer to results obtained about 5 min after completion of the preparatory procedures the shaded bars to those obtained after 1 h of rest, and the black bars to data obtained about 5 min after completion of the preparation when effective β-adrenergic blockade was also instituted. It can be seen that the dynamic myogenic constrictor response was small and the microvascular tone low just after completion of the surgery (white bars) compared to the situation after 1 h of rest (shaded bars). During the resting period

the ventricular spaces to the vital centers The plateau in the dose-response curve of h r at higher doses is probably due to the limitation set by the maximal heart rate The increased r r in response to TRH was followed by a decrease in tidal volume and hence we did not find any change in respira tory minute volume Respiratory feed-back mechanisms appear to work under influence of TRH The occurrence of a positive cornea reflex after TRH infusions might suggest that the changes observed in m b p h r and r r are primarily due to the antagonizing effect of TRH on narcosis so that the effects observed are connected with wakening The great magnitudes in increases of b p h r and r r after TRH administration however suggest that TRH might directly stimulate medullary vital centers Horita et al (1977) suggested that some central effects of TRH are mediated by catecholamines The physiological phenomena affected by TRH infusions are under the influence of medul la oblongata and spinal cord and this is in accord ance with the high TRH content of these areas Nicoll (1977) showed an excitatory action of TRH on frog spinal motoneurones and there are reports of an inhibitory action on supraspinal neurones (Dyer et al 1974 Renaud et al 1975) This together with the wide distribution and profound effects suggest that TRH could act as a neurotransmitter

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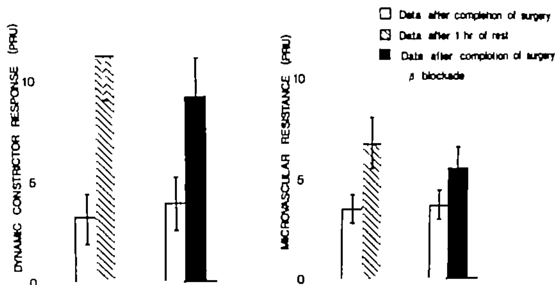


Fig 1 Mean data ±S E for dynamic myogenic microvascular constrictor responses to increase of vascular transm pressure by 30 mmHg applied at the rate of 2.5 mmHg/s and for basal microvascular tone in skeletal muscle as recorded immediately after completion of surgery (white bars) after 1 h of rest (shaded bars) and after β-adrenergic blockade administered few minutes after completion of surgery (black bars) *n* = 4 The results indicate a strong β-adrenergic inhibitory interference with myogenic reactivity and basal vascular tone caused by experimental intervention.

there was a gradual recovery of both variables and plateau values were reached after these 60 min Note however that β-adrenergic blockade in the early period led to much quicker recovery of dynamic myogenic reactivity and microvascular tone (black bars)

The static myogenic constrictor response to constant increased transmural pressure (+30 mmHg) was small compared to the dynamic response but it showed a similar pattern its amplitude averaged 0.5 PRU when elicited just after completion of surgery and rose gradually to a mean value of 1 9 PRU after 1 h of rest β-adrenergic blockade in the early observation period instituted quick recovery of the static response from 0.5 to 1 4 PRU

The described dynamic myogenic microvascular constrictor response was always reflected a a simultaneous corresponding increase in total regional vascular resistance The more proximal arterial vessels and the large veins seemed to lack dynamic myogenic reactivity almost entirely

DISCUSSION

The present results demonstrate impaired myogenic vascular reactivity and decreased vascular tone in skeletal muscle in the early period after completion of the surgery and a gradual recovery towards a steady state within about an hour of subsequent rest. These findings indicate the presence of sor inhibitory factor(s) which gradually disappears du ing extended rest and therefore most likely w activated during the surgical and other experiment intervention The demonstrated much quicker r covery after β-adrenergic blockade strongly su gests that this inhibitory effect to a major exte is caused by a β-adrenoceptor stimulation (catecholamines This interpretation is compati with previous observations (Lundvall 1976, Lu vall & Hillman 1978) of inhibitory β-adrenerg microvascular effects of adrenaline and noradr aline infusions The present results indicate th such inhibitory influence also specifically interfer with myogenic reactivity to transmural pressur stimuli Some other inhibitory factor howeve might also be involved since the recovery after β blockade was not entirely complete (Fig. 1)

The β-adrenergic interference with myogen reactivity was present despite attempts in this stu to minimize the experimental trauma such an effe might therefore be more profound and longlast if severe trauma is involved It is quite likely th such an influence can have contributed to poor variable myogenic reactivity reported in the earl literature A more detailed investigation in progre (Grände 1979) strongly suggests that the sy patho-adrenal control system via its β-adrenerg link in important respects can modulate myogen reactivity also under physio l conditi

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cemaker properties of mammalian Purkinje cells

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kground discharge recorded extracellularly n Purkinje cells has been attributed to continu- secretion of excitatory transmitter from the nerous synapses of parallel fibers (Eccles et al. 7). Purkinje cells remained spontaneously active vitro however (Yamamoto 1973) even when aptic transmission was blocked with manganese cobalt ions (Hounsgaard & Yamamoto 1978) s preliminary results presented in the following ount indicate that spontaneous spike activity is inherent property to the Purkinje cell membrane is conclusion is based in intracellular recordings m 10 Purkinje cells on thin cerebellar sections m Guinea pigs prepared and maintained as de- bed by Yamamoto (1973)

The composition of the medium was (mM): NaCl, KCl, 5 KH_2PO_4, 1,25 $MgSO_4$, 2,0 $CaCl_2$ 2 $NaHCO_3$, 26 and glucose 10 In this medium synaptic transmission was preserved. In all cells stable intracellular recording could be maintained for at least 20 min, in 3 cells for more than 1 h.

The neurons discharged regularly after impalement with a microelectrode (Fig. 1 A). The spike frequency varied from 5 to 50 Hz in different neurons but was constant for long periods of time in any given cell. Most cells were continuously active but some were silent in periods which occurred at constant intervals. These results are in agreement with those obtained with extracellular recording in vivo (Bell & Grimm 1969 Latham & Paul 1971) and in vitro (Yamamoto 1973).

A bridge circuit was used to pass current through the recording electrode.

The spike frequency increased during a depolariz

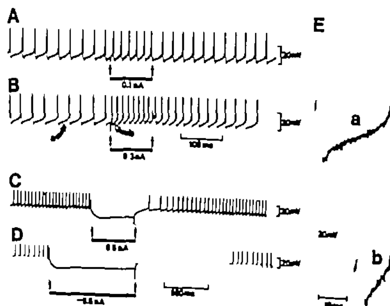


Fig. 1 Effects of current injected in spontaneously active Purkinje cells. (A) Spike frequency increased moderately when +0.1 nA was injected (injection time indicated by the horizontal bar). (B) A larger increase was produced when +0.3 nA was injected. The voltage trajectories marked by curved arrows are shown in higher magnification in E.a and E.b Action potentials were abolished when −0.5 nA was injected. When the current was terminated after 500 ms (C) the spike frequency was slowed during the next 600–800 ms. Injection of −0.5 nA for 1 s (D) was followed by depolarizing overshoot and spontaneous action potentials reappeared after more than 1 s.

ing current pulse (Fig 1 A) This increase was linearly related to the amplitude of the current pulse except for a frequency fall off sometimes seen at high current strengths (>1 nA) As the spike frequency increased the flat part of the voltage trajectory became shorter until the initial and the late steep part finally fused (Fig 1 B and 1 E)

The generation of spontaneous action potentials stopped during a hyperpolarizing current pulse (Fig 1 C) When the pulse terminated after more than 100 ms there was a delay until the first action potential and the first few interspike intervals were prolonged (Fig 1 C) The delay and the interspike interval increased with increasing duration and magnitude of the hyperpolarizing current In some cells the initial part of the repolarizing phase after a long hyperpolarizing pulse was very prominent giving rise to a depolarizing overshoot (Fig 1 D)

The generation of repetitive action potentials in Purkinje cells described above cannot be accounted for by the Hodgkin-Huxley equations (Jack Nobel & Tsien 1975) However they are fully accounted for by models describing repetitive firing in snail neurons (Connor & Stevens 1971 Neher 1971) In addition to the voltage dependent conductance changes giving rise to the inward and outward current during an action potential these models include a voltage sensitive outward current channel governing the membrane potential during the interspike interval The overshoot (Fig 1 D) and the reduction of firing frequency (Fig 1 C) immediately after a hyperpolarizing current pulse of long duration are specifically predicted by the model (Connor & Stevens 1971) This additional outward current may well be mediated by potassium or chloride ions From a different point of view the possibility of an additional potassium current mechanism in Purkinje cells has recently been discussed (Nicholson et al 1978)

In conclusion repetitive firing in Purkinje cells in vitro is determined by the properties of the membrane Models accounting for repetitive fi[ring] in certain snail neurons may also account for repetitive firing of Purkinje cells

Detailed investigation of repetitive firing in Purkinje cells is in progress.

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omerular capillary pressure in the rat

idation of pressure measurement through corticotomy

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recent years many studies have been reported on illary pressure measured by direct puncture of erficial glomeruli in rats (for ref see Tucker & ntz 1977). However uncertainties still remain as how representative the punctured glomeruli are, ause (1) superficial glomeruli accessible to mic uncture are found only in special rat strains ener et al. 1971 Kallskog et al. 1975) (2) the ssure in the few accessible glomeruli might differ m that in the great majority of deep glomeruli not ble on the kidney surface. To get around these blems we recently introduced corticotomy a cedure in which a lense-shaped slice of the cor is cut out, exposing a large number of glomeruli different depths of the cortex (Aukland et al. 77). Only indirect evidence was presented to w that this admittedly traumatic procedure does change the glomerular pressure More direct dence is presented in the present study showing t the pressure measured in exposed glomeruli s not differ from those measured through the act renal capsule Attempts were also made at al staining with Alcian Blue to make more meruli visible and thereby accessible to trans- sular measurements

Experiments were performed on two strains of male Wistar rats weight 150–235 g. One strain s obtained from K E. Mollegaard Ejby Den- rk (W-Mo) and the other strain was the mutant unich strain from S Ivanovas GMBH & Co sslegg im Allgau W-Germany (W Munich) The mals were fed standard rat pellets Food, but not ter was withdrawn in the afternoon the day be- the experiment All rats were anesthetized with ctin (Promonta, Hamburg) given as a single i p ection of 120 mg/kg b w The rats were cheotomized and placed on a heated operating le oesophageal temperature being controlled at 5–37.5°C A femoral artery and a vein were theterized for arterial pressure recording and for travenous saline infusion (1 ml/h). In W Mo rats, a polyethylene tube (PE 10) was placed in the upper abdominal aorta via a femoral artery for infusion of Alcian Blue. The left kidney was prepared for micropuncture as previously described (Aukland et al. 1977) and glomerular capillary pressure (P_G) was measured through the intact renal capsule (P_{GT}=transcapsular) The kidney was thereafter corticotomized by cutting off a 1–2 mm thick lense shaped slice with a diameter of about 5 mm (Aukland et al 1977) and glomerular pressure measured through the corticotomy (P_{GC}) The depth of the punctured glomeruli relative to kidney surface varied from 0 1 to 0 9 mm, with almost 70% of the punctured glomeruli located 0.1 to 0.3 mm under the capsule Micropuncture was performed with glass pipettes with sharpened tip of 1–2.5 μm diameter Measurements were obtained by means of servocontrolled counter pressure (Wiederhielm et al. 1964 Aukland et al. 1977).

From 2 to 20 superficial glomeruli could be observed on the intact dorsal aspect of the kidney in

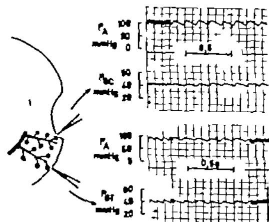


Fig 1 Original recordings of arterial (P_A) and glomerular capillary pressure measured through intact capsule (P_{GT}) and through corticotomy (P_{GC}). Left: Location of micropuncture in corticotomized kidney

Table 1 *Glomerular capillary pre sure (mmHg) measured through intact capsule* (P_{GT}) *and in corticotomy* (P_{GC}) *Mean ±S E*
n = number of animals Number of glomeruli in parentheses, P = mean aortic pressure AB = Alcian Blue

Strain	*n*	P_{GT}	P_{GC}	P
W Mø	14	47.3±2.2 (20)	46.3±2.4 (12)	110.9±2.6 (32)
W Mø after AB injection	4	47 4±3 6 (5)	52.0±6.5 (4)	97 7±4.8 (9)
W Munich	14	49 1±1 6 (21)	50.3±1 8 (17)	107.2±2.6 (38)

all W Munich and in 13 of the W Mø rats In 10 W Mø rats which had no detectable surface glomeruli bolus injection of 0 1–0 2 ml Alcian Blue 8GX Gurr (0 5–1 % in saline solution) was given in the abdominal aorta.

Satisfactory measurements were obtained both in 1–3 superficial glomeruli before corticotomy and in 1–4 glomeruli in the corticotomy in 9 out of 14 W Munich and in 11 out of 17 W Mø rats In the remaining 11 rats only P_{GT} or P_{GC} measurements were obtained Original recordings of P_{GT} and P_{GC} in the same kidney are shown in Fig 1 As evident from Table 1 capillary pressure in glomeruli punctured through the intact kidney capsule showed no difference from pressure in glomeruli in the corticotomy The individual paired measurements of P_{GT} and P_{GC} from a total of 11 W Mø rats (32 glomeruli) and 9 W Munish rats (32 glomeruli) are shown in Fig. 2. In 2 W Mø rats measurements of P_{GT} were repeated after corticotomy In these rats P_{GT} was 60 and 41 mmHg before corticotomy compared to 54 and 40 mmHg after corticotomy

In accordance with previous results (Aukland et al 1977) P_G showed no correlation to spontaneous intra- and interindividual variations in aortic pressure

I a. injection of Alcian Blue in 10 W Mø rats visualized up to 15 glomeruli on the intact kidney surface However a sudden shrinkage discolouring of the kidney and a gradual decline in P_A were observed in some rats especially after larger doses In the 4 rats included in Table 1 no such signs of toxicity were observed and glomerular pressure seemed to be unchanged

The absolute glomerular capillary pressures obtained in the present study need some comments The average P_G observed in W Munich rats agrees fairly well with those reported for this strain by other investigators (Tucker & Blantz 1977) However the similar average P_{GC} of 46.3±2.4 mmHg in W Mø rats is about 12 mm lower than what we have previous reported in purportedly the same st[illegible] The use of only females in the present study [illegible] not seem to explain the difference (Aukland e[illegible] 1977) Since we are not aware of any chang[illegible] methodology the difference is probably due [illegible] ferences among batches of rats In fact, the ba[illegible] W Mø rats used in the present study had [illegible] many visible glomeruli as the W Munich rats, many more than we previously observed [illegible] rats from Møllegaard While the supplier [illegible] the possibility of "contamination from the [illegible] Ivanovas Munich strain it is well known that [illegible] are large variations within the socalled W [illegible] strain which is not genetically well defined [illegible] Erichsen Statens Institutt for Folkehelse, [illegible] Personal communication)

The present study strongly supports our pre[illegible]

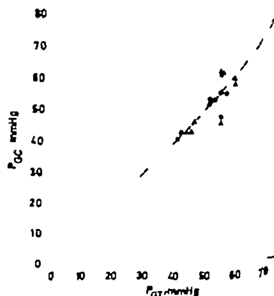


Fig 2 Paired measurement of glomerular p[illegible] punctured through intact kidney capsule (P_{GT}) through corticotomy (P_{GC}) ● Wistar Møllegaard ▲ Wistar Munich rats.

...ression that corticotomy does not greatly influ... e glomerular capillary pressure. Admittedly ...h only one or two exceptions, the glomeruli ...ctured in the corticotomy were too deep to be ...le through the intact capsule and might in ...ory have had a different pressure before cor...itomy. The previous finding of similar P_{GC} at ...ous depths of the cortex makes this unlikely. In ... case, we have shown that "control" pressures ...sured in corticotomy agree well with transcap...r measurements which would therefore seem ...ive a good estimate of average glomerular capil... pressure. However the observations do not ...lude the possibility of different responses to ...iological or pharmacological stimuli in deep ... superficial glomeruli.

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Release of ^{3}H nucleosides from ^{3}H-adenine labelled hypothalamic synaptosomes

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[^{3}H]adenine was taken up by a crude hypothalamic synaptosomal fraction and incorporated into mainly nucleotides. The synaptosomes were superfused and after the initial washout a steady fractional release rate of 0.5–1 % of the content/min was found. Electrical pulses (2 ms, 50 Hz, 10–20 mA, 4 min) and veratridine (10 μM, 4 min) induced Ca^{++}-dependent increase in purine release rate. K$^+$ (30 mM 4 min) caused a largely Ca^{++}-independent increase. Most of the released material co-chromatographed with adenosine inosine and hypoxanthine, while little or no nucleotide material was detected. Release of endogenous adenosine inosine and hypoxanthine was detected by high performance liquid chromatography. However following hypo-osmotic shock most of the released material was in nucleotides. The removal of glucose from the medium increased the fractional release rate 2 3 fold. Histamine, acetylcholine and glutamate were without effect. High amounts of noradrenaline caused an EGTA-inhibited release of purines which was unaffected by propranolol or phentolamine. It is suggested that purines are released from neuronal structures and that the release reflects increased energy consumption and/or decreased energy production.

Key words: Adenosine ATP depolarization energy metabolism transmitter release, calcium dependence

There is considerable evidence that purine compounds may influence activity in the central nervous system. Thus adenosine and several adenosine analogues depress firing rate in rat cerebral and striatal neurons (Phillis & Edstrom 1976, Siggins et al. 1976) and in neurons of the guinea pig olfactory cortex (Kuroda et al 1976). The effect is antagonized by methylxanthines and potentiated by drugs that inhibit adenosine inactivation (Phillis & Edstrom 1976, Siggins et al 1976 Kuroda et al. 1976). Since such inhibitors are effective per se it has been suggested that endogenous adenosine is present in sufficient amounts to exert physiological effect (Phillis & Edstrom 1976).

Adenosine also has effects on peripheral nerves. Thus inhibition of noradrenaline release (Hedqvist & Fredholm 1976) acetylcholine release from cholinergic nerve endings in the gut (Vizi & Knoll 1976, Sawynok & Jhamandas 1976, Gustafsson, Hedqvist & Fredholm 1977) and in skeletal muscle (Ginsborg & Hirst 1972) has been shown or strongly implied. These effects are also antagonized by theophylline (Hedqvist, Fredholm & Ölundh 1978 Vizi & Knoll 1976, Sawynok & Jhamandas 1976, Gustafsson et al 1978). Since adenosine-like material is released from these tissues by nerve stimulation (e.g. Fredholm 1976 Fredholm & Hedqvist 1978 Su Bevan & Burnstock 1971) it has been suggested that adenosine could act as an endogenous modulator of transmitter release (e.g. Hedqvist & Fredholm 1976, Sawynok & Jhamandas 1976).

The studies of Kuroda et al. (1976) strongly suggest that the central effects are mediated over cyclic AMP. Adenosine and adenosine derivatives are known to be potent activators of cyclic AMP formation in guinea pig cortex (Sattin & Rall 1970) as well as in other brain regions (cf Daly 1975). This effect appears to be due to the direct stimulation of adenylate cyclase as shown in homogenates of mouse neuroblastoma cells (Blume & Foster 1975 1976) rat striatum (Prémont et al 1977 Fredholm 1977) and guinea pig and monkey hypothalamus

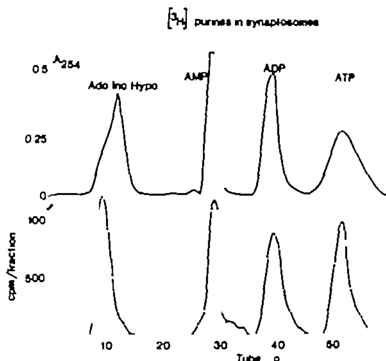


Fig 1a [H]-purines in synaptosomes. Synaptosomes were incubated with [³H]adenine for 15 min, washed twice and proteins precipitated with 0.4 M perchloric acid Chromatography of neutralizated protein-free supernatant on a 0.8×?0 cm Dowex 1×2 column The initial 16 fractions were eluted with water The remaining fractions were eluted with a concave gradient of HCl (0–0 4 M) The upper panel represents the absorbance of added carriers (adenosine inosine hypoxanthine AMP ADP and ATP). The lower panel shows the radioactivity in the collected fractions.

(Ahn & Markman 1977) The effect of adenosine on cyclic AMP is blocked by theophylline and other methylxanthines and enhanced by various inhibitors of adenosine inactivation (Sattin & Rall 1970 Daly 1975 Blume & Foster 1975 Ahn & Makman 1977 Fredholm 1977 Prémont et al 1977) Adenosine is rapidly taken up by neuronal stru tures in vitro (Pull & McIlwain 197?) and in vivo (Schubert & Kreutzberg 1974) It is incorporated into the nucleotide pool (Pull & McIlwain 197?) and is subject to axonal transport (Schubert & Kreutzberg 1974) and is released following excitation from brain slices (Pull & McIlwain 197? Schubert & Kreutz berg 1974) and guinea pig neocortical synaptosomes (Kuroda & McIlwain 1974)

In the present study we have with the above mentioned results as a background studied the release of [³H]purines from hypothalamic synaptosomes labelled with [³H]adenine A recently described superfusion technique (Mulder et al 1975) with Sephadex G 15 as a support for the synaptosomal preparation was used in these studies.

MATERIAL AND METHODS

The procedure to isolate and superfuse the crude synaptosomes closely followed that described by Mulder et al (1975) Male Sprague-Dawley rats (180–220 g) were decapitated using a guillotine The brains were rapidly dissected out and the hypothalami washed in Krebs-Ringer bicarbonate buffer Hypothalami from three rats were homogenized in 5 ml 0 32 M sucrose by means of a Potter Elvehjem glass homogenizer with a rotating teflon pestle The homogenates were centrifuged at 600×g One ml of the supernatant was diluted 5 times with a Krebs-Ringer bicarbonate medium of the following composition (mM) NaCl (118) KCl (4 85) $MgSO$ (1 15) KH_2PO (1 19) $NaHCO_3$ (25) $CaCl_2$ (0 25) glucose (11 1) The buffer was continuously gassed with 95% O and 5% CO_2 and the pH was 7.3–7 4 After preincubation for 5 min at 37°C in a shaking waterbath 10 µCi -[³H]adenine (23 Ci/mmol New England Nuclear) or ,8-[³H]adenosine (30.4 Ci/mmol New England Nuclear) was added and the incubation continued for 10 min.

The labelled particulate material was collected by centrifugation for 10 min at 600×g After resuspension an aliquot of the crude synaptosomal preparation was applied to each of the four chambers of the superfusion system

Each chamber consisted of plastic 1 ml syringe At the bottom plug of cotton was inserted On top of that a layer of Sephadex G 15 (Pharmacia, Uppsala) of ?0 µl

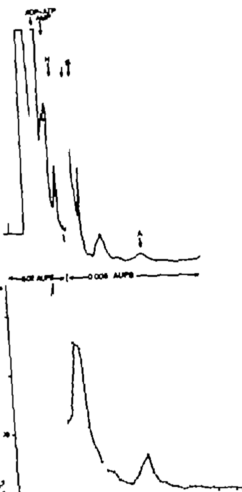


Fig. 1b. Chromatography of 25 µl neutralized protein-free extract on high pressure liquid chromatography as described under methods. The upper curve represents absorbance at 254 nm (the first 5 min at 0.02 AUFS, thereafter at 0.005 AUFS). The lower curve represents radioactivity in fractions continuously collected at 30 s intervals. The arrows indicate the positions of reference compounds run on separate occasion. H hypoxanthine, I inosine, G guanosine, A adenosine.

...olume ... as placed. The synaptosomes were layered on top of the Sephadex. A movable plunger pierced by plastic tubing to allow the medium to enter the chamber was placed approximately 2 cm above the synaptosomal layer. The chambers ... re surrounded by water kept at 37°C. The medium was superfused at the rate of 0.3 ml/min by means of ... peristaltic pump (Gilson Minipuls HP-4). 2 min fractions were continuously collected in plastic tubes. After the rate of overflow had reached ... steady level the synaptosomal preparation was depolarized for ... period of ... min in either of 3 ways. ( ) the potassium level was increased to 30 mM (... ith ... compensatory decrease in NaCl). (b) veratridine (10 µM) was included in the superfusing buffer solution and ( ) square pulses of 2 ms duration were delivered at ... rate of 50 Hz between ... or coil electrodes located at a distance of about 2 cm. Total applied voltage was 50 V and the resultant current was 10–20 mA.

At the end of the experiment the entire synaptosomal bed with its support was expelled into counting vials and the radioactivity determined. Release rates are given as fractional release rates.

Separation of treated purines. The H-purines were chromatographically separated in either of 3 ways: ( ) ion-exchange chromatography on Dowex 1 X 2 (200–400 mesh, 0.8×20 cm) as described previously (Fredholm 1976); (b) thin-layer chromatography on silicic acid (DC fertigplatten, Merck) using butan (1)-ol, ethylacetate, methanol NH_4OH (7 4 3 4) (Pull & McIlwain 1972); ( ) thin-layer chromatography on polyethyleneimine cellulose (DC-alufolien, Merck), essentially as described by Böhme & Schultz (1974) using development with 50 mM HAc, distilled water and 1 M LiCl, successively. In either system appropriate carriers were included. In the column chromatographic system the evolution of the carriers was followed by continuous registration of the ultraviolet absorption at 254 nm with an ISCO model UA5 absorbance monitor. The reference spots on the thin-layers were identified under ultraviolet light.

Separation of purine compounds using reversed phase high pressure liquid chromatography (HPLC) was performed on some occasions. The system consisted of Waters model 600 Solvent delivery system ... UK6 universal injector ... µ-Bondapak C18 reversed-phase column and a model 440 Absorbance monitor. Although nominally the maximal sensitivity of the latter is 0.005 absorbance units full scale deflection (AUFS) it is possible to increase sensitivity 2-fold further by adjusting the recorder input from 10 mV to 5 mV without obtaining an unacceptable background. The chromatograms were developed isocratically using 5 mM ammoniumphosphate (pH 6.0) containing 15% methanol as the running phase. The identity of the relevant peaks was confirmed by comparisons with standards and by selective changes induced by treatment with the enzymes adenosine deaminase and nucleoside phosphorylase.

The radioactivity in the superfusates, the eluted fractions and/or the spots on the chromatograms was counted in ... liquid scintillation spectrometer (Intertechnique) using 3.94 g 2,5-diphenyloxazole (Packard) and 0.06 g p-bis-(o-methylstyryl) benzene (Kent Laboratories) per litre of toluene: triton X 100 (2 1) as scintillator. Quenching was monitored by external standardization and counting efficiency averaged 38%.

ATP-determination. ATP levels in ... synaptosomal preparations was determined by the luciferine-luciferase method essentially ... s described by Li & White (1977) except that ... Packard Scintillation Spectrometer was used to measure the induced luminescence. The synaptosomes were deproteinized with 0.6 M PCA and the protein pellet was used for protein determination by the Lowry-method using bovine serum albumin as standard.

Measurement of cyclic AMP. The cyclic AMP content of the effluent from the synaptosomal bed was measured

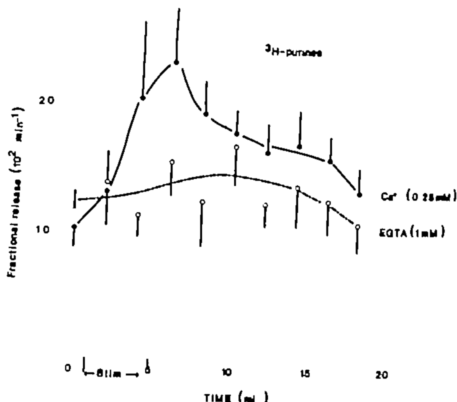


Fig 2 Effect of electrical stimulation as the release of [³H]purines from a synaptosomal bed Results are given as mean ±S E ●—● 0.25 mM Ca (n=6) O– –O 0 mM Ca⁺⁺ 1 mM EGTA (n=4)

by a competitive binding assay as described by Brown et al (1972) using binding protein prepared from bovine adrenal cortex

Drugs The drugs used came from the following suppliers noradrenaline adrenaline and L-isoprenaline (all as bitartrates) L-glutamate acetylcholine chloride histamine adenosine adenine adenosine 3',5'-monophosphate (cyclic AMP) adenosine 5' monophosphate (AMP) adenosine 5'-diphosphate (ADP) and adenosine 5' triphosphate were supplied by Sigma Inosine was from Boehringer Mannheim veratridine from Aldrich Chemicals, bis (aminoethyl) glycolether N,N' N',N¹-tetraacetic acid (EGTA) and hypoxanthine from Merck, Darmstadt propranolol (Inderal®) from ICI and phentolamine (Regitin®) from CIBA Other chemicals were reagent grade from various chemical suppliers

RESULTS

Immediately following labelling aliquots of the incubates were washed twice and deproteinized Under these conditions more than 65% of the total radioactivity taken up was retained as nucleotides approximately equally distributed as AMP ADP and ATP (Fig. 1*a*) Of the non-nucleotide material the majority was inosine and adenosine their relative proportions being approximately 8 1 (Fig. 1*b*)

The specific radioactivity of adenosine was approximately 250 dpm/pmol and of inosine dpm/pmol

An aliquot (0 1-0 25 mg protein in 50 µl) of the synaptosomal fraction containing 40-70 nCi [³H] label was applied to each of 4 perfusion chambers During the first 15 min of washing approximately one third of the radioactivity was released. The released material was in the form of nucleosides and bases and therefore corresponds to the non-nucleotide fraction after labelling. Thereafter the rate of ³H-overflow was 0 8-1 $\times 10^{-4}$ of the total content per minute 35 min after the start of superfusion a steady rate of 0 35–1 2×10^{-4} of the total content per minute was reached and remained quite steady at this level for the remainder of the 50–80 min superfusion period This fractional release rate is similar to that reported for guinea pig cortical synaptosomes (Kuroda & McIlwain 1973)

The ATP content of the crude synaptosomal preparation was 2 08±0 08 nmol/mg protein (n = This value is very close to that reported for whole brain synaptosomes by Li & White (1977). It proved essentially impossible to determine the protein content of the synaptosomes after the superfusion

[ta]ble 1. Relative distribution of radioactive [p]urines after hypo-osmotic shock or veratridine

[Sep]aration by chromatography on silicic acid or PEI-cel[lulo]se (see Methods). The fractional release rate following [hyp]o-osmotic shock averaged 3.2%/min, while following [vera]tridine it [w]as 1.6%/min

	Relative distribution (% of total radioactivity)			
	Nucleo-tides	Ino	Hypo	Ado
[Hy]po-osmotic				
[Si]O₂	72	6	20	3
[P]EI	79	5	20	6
[Ve]ratridine				
[Si]O₂	4	22	22	19
[P]EI	6	29	28	28

[treat]ment and therefore only the total ATP content [cou]ld be determined. In one experiment the ATP [con]tent in 4 samples of the synaptosomal prepara[tion] before layering it on the Sephadex column was [0.]35±0.02 nmol and after 110 min of superfusion [(in]cluding t[w]o stimulations with [v]eratridine) it was [0.]39±0.04 nmol. In another experiment the cor[res]ponding values were 0.37±0.05 and 0.30±0.02 [nm]ol, respectively. Thus, there was no e[v]idence for [an]y marked loss during the experiment. By contrast [th]e endogenous adenosine levels decreased sub[st]antially during superfusion. Initially 50±10 pmol [w]as found while the levels at the end were close to [th]e detection limit or about 10 pmol/synaptosomal [be]d. These findings offer further support for the [c]ontention that the initial loss during superfusion [w]as comprised of nucleoside rather than nucleotide [m]aterial.

The rate of ³H-overflow was enhanced by electr[ic]al field stimulation (Fig.) increasing the K[⁺] con-tent of the buffer to 30 mM (not shown) and by veratridine (Fig. 3). The release induced by electrical pulses was calcium-dependent (Fig.) as reported earlier for guinea pig cortical synaptosomes (Kuroda & McIlwain 1974). The total amount of ³H-radioactivity released by the electrical stimulation (the basal release substrate) was $6.5\pm1.2\times10^{-4}$ of the tissue content (mean±S.E.) in the presence of 0.25 mM Ca^{++} and $1.5\pm0.6\times10^{4}$ of the tissue content in the absence of Ca^{++} but presence of 1 mM EGTA ($p<0.02$ by Student's t test for unpaired variates).

Changing the buffer solution to 30 mM K[⁺] 93 mM Na[⁺] for 4 min caused a release which showed only marginal calcium dependence ($2.8\pm0.2\times10^{-4}$ in the presence and $2.2\pm0.2\times10^{-4}$ in the absence of Ca^{++}). By contrast, the single injection of 0.32 ml of 0.8 M KCl produced a Ca-dependent release in cortical synaptosomes (Kuroda & McIlwain 1974). Superfusion of the synaptosomal bed with 10μM veratridine for 4 min produced a clearly calcium-dependent release (Fig. 3). In the presence of 0.25 mM Ca^{++} $4.2\pm0.3\times10^{-}$ (mean±S.E.) of tissue content was released, in calcium-free buffer containing 1 mM EGTA $1.8\pm0.6\times10^{-4}$ of the tissue content was released ($p<0.01$ by Student's t-test for paired [v]alues). The calcium-dependence was decreased when 50 μM veratridine was used.

The radioactivity released in response to either agent was mainly in the form of nucleosides as demonstrated by TCL in two systems. Fig. 4 shows the distributions of superfusate radioactivity during the first and second phase of purine release following veratridine. It may be seen that during the first phase of release (4–8 min following the administration of the veratridine pulse) the dominating product was adenosine. During the second part of the release curve (8–16 min after the start of the vera-

[T]able [2]. Release of endogenous purines f[r]om isolated rat hypothalamic synapt[o]somes

[1]00 μl of the perfusate [w]as injected into [a] HPLC (Waters) and the purine content detected by absorbance at 254 nm [(]0.005 AUFS). Stimulation was induced by the administration of 10 μM veratridine for 4 min

	pmol/min			
	Adenosine	Inosine	Hypoxanthine	Σ
Before stim. I	1.6	3.6	1.6	6.8
10 min post stim. I	2.2	4.2	2.7	9.1
Before stim. II	1.3	3.6	1.1	6.0
10 min post stim. II	1.3	4.2	1.9	7.4

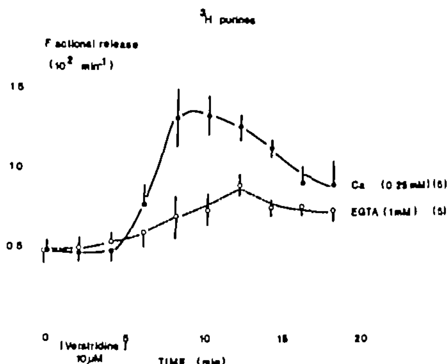


Fig 3 The effect of veratridine (10 μM) on the release of [H]purines from a synaptosomal bed Mean ±S E ●—● 0 25 mM Ca (n=5) O---O 0 mM Ca 1 mM EGTA

tridine pulse) inosine and hypoxanthine had increased in proportion In the silicic acid chromatography system there was radioactivity in a fraction corresponding to nucleotides (i e at the origin) Since little or no nucleotide material was found in the PEI-cellulose chromatography it may be assumed that what appears to be nucleotides on the SiO_2 TLC represents artefactual adsorption of non-nucleotide material at the site of application (Fig. 4 Table 1) There was however some evidence of cyclic AMP release as judged by the PEI cellulose chromatography Therefore the cyclic AMP content of the superfusion was determined directly in 4 expts In neither of them could release of cyclic AMP by K or veratridine be detected however

An essentially similar distribution as that found following veratridine was found following release by electrical stimulation On the other hand the radioactivity release by hypo-osmotic shock (H O-injection) was composed mainly by nucleotides as judged by SiO_2 and PEI-cellulose chromatography (Table 1)

The release of endogenous purines was detected by HPLC (Table 2) The rates of release were low (a few pmol/min) and the corresponding concentrations are approximately 8 nM (adenosine) 20 nM (inosine) and 9 nM (hypoxanthine) It is apparent that the rate of release was quite constant during the experiment This provides support for the constancy as determined by release of radioactivity It is interesting to compare the relative proportions of the three compounds adenosine inosine and hypoxanthine in Tables 1 and 2 Based on radioactivity (Table 1) the amounts released are approximately equal However endogenous inosine was approximately 2.5 times higher than adenosine and hypoxanthine (Table 2). Interestingly the same ratio was found in terms of specific radioactivity in the synaptosomal preparation (Fig 1 *b*) The fact that endogenous inosine was higher than could be expected from the radioactive inosine could possibly be explained by inosine formation from guanosine and guanine nucleotides that would probably not be labelled by [^{3}H]adenine

It is known that synaptosomes release a number of putative transmitter substances upon electrical stimulation addition of veratridine or depolarization with high potassium For example the release of dopamine and noradrenaline (e g Blaustein et al

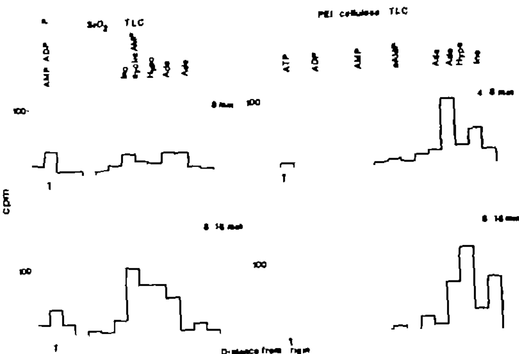


Fig. 4. Thin-layer chromatography of purines released from synaptosomal bed 4–8 min (upper part) or 8–16 min (lower part) following veratridine (10 μM). Further details see text.

972, Mulder et al. 1975), serotonin (Mulder et al 1975), glutamate (Takagaki 1976), GABA (Levy et al. 1973), and acetylcholine (Blaustein 1975) has been demonstrated. It was therefore possible that the release of [^{3}H]adenosine and other [H]purines was indirect and due to the actions of other transmitter substances. Therefore the effect of some putative transmitters in the hypothalamus was tested. In Fig. 5 is shown that very high concentrations of noradrenaline is capable of releasing ^{3}H radioactivity. In control experiments where ^{3}H]noradrenaline was injected it was found that the peak concentration of noradrenaline following injection of 10^{-4} moles was 4×10^{-6} M. The noradrenaline-induced release was inhibited by 1 mM EGTA, but could not be inhibited by either propranolol (10^{-} M) or phentolamine (10^{-4} M).

In other experiments glutamate (10^{-4} M), acetylcholine (10^{-4} M) and histamine (10^{-4} M) were without significant effect.

Effect of hypoxia and glucose deprivation

Hypoxia is known to increase the level of adenosine in several tissues including guinea pig neocortical slices (Newman & McIlwain 1976). Therefore the effect of bubbling the superfusions buffer with N_2–CO_2 (95% 5%) instead O_2–CO_2 (95% 5%) was tested. It may be seen from Fig. 6, which illustrates one typical experiment that exchanging O_2 for N_2 had no significant effect on the rate of [^{3}H]purine release. The pooled data confirm this lack of effect. An explanation for the apparent lack of effect of hypoxia is that the plastic tubing and the plastic chambers are rather freely permeable to gases. This is shown by the fact that the pO_2 in the O_2-bubbled buffer is 340 mmHg (310–365) while the pO_2 of the buffer emerging from the chamber is 193 mmHg (160–215). The corresponding values for pCO_2 are 29.5 (28.5–32) and 4 (20–25.5) mmHg. As a consequence of the loss of CO_2 from the buffer the pH of the buffer leaving the chamber is 7.4 (7.36–7.42) while the pH of the buffer entering the tubing and chambers is 7.31 (7.295–7.34). Thus, atmospheric *oxygen might penetrate the tubing and the chamber* in sufficient amounts to prevent acute hypoxia of the synaptosomes. The administration of antimycin, a well-known inhibitor of oxidative phosphorylation, caused a very clearcut increase in [^{3}H]purine release. Moreover omission of glucose from the *medium significantly* enhanced the basal rate of

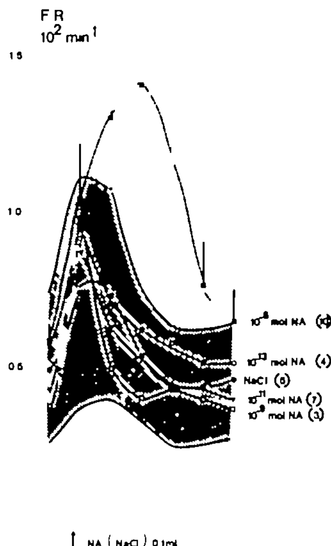


Fig 5 Release of [^{3}H]purines from a synaptosomal bed by the injection of noradrenaline (10^{-} -10^{-} mol in 0.1 ml) ●——● Control injection of 0 1 ml NaCl The hatched area represents ±1 S D ○– –○ 10 $^{-13}$ mol NA (n=4) ▼– –▼ 10^{-} mol NA (n=7) □– –□ 10^{-9} mol NA (n=3) ■– –■ 10 mol NA (n=10) Vertical bars represent S E.

[^{3}H]purine release both in the presence and absence of oxygen In the presence of glucose the fractional release rate was 0 67±0 09 (n=12 O_2-gassed) and 0 58±0 03 (n=8 N_2-gassed) In the absence of glucose in the medium the correspond ing values were 1 18±0 15 (n=6) and 1 42±0 08 (n=4)

DISCUSSION

The present results show that [^{3}H]purines may be taken up and released from rat hypothalamic synaptosomes as shown earlier for guinea pig corti cal synaptosomes (Kuroda & McIlwain 1974). The fractional release rate agrees with that reported by these authors for synaptosomes but is higher than that found by the same group using guinea pig neocortical slices (Pull & McIlwain 1972). The difference between synaptosomes and slices could be due either to that the synaptosomes are more fragile than intact slices or to that reuptake of labelled purines is a more significant process in slices. The finding that adenosine markedly enhances [^{3}H]purine release from slices may be taken as evidence that reutilization is significant in the slice preparation (Pull & McIlwain 1973) and hence that the latter explanation is correct

Electrical stimulation veratridine and depolarization by potassium all increased the release of [^{3}H]purines from the synaptosomal preparation Synaptosomes maintain a gradient of potassium and sodium (cf Blaustein 1975 Li & White 1976). Upon depolarization there is a rapid change in ion composition including an increase in calcium content (Blaustein et al 1977 Blaustein 1975). There is also a rapid fall in ATP content (Li & White 1976). A fall in synaptosomal ATP content is also seen following inhibition of cellular energy production by sodium cyanide (Li & White 1976) This fall in ATP may be an explanation of the increase in [^{3}H]purine release found in the present study The finding of a significant increase in [^{3}H]purine release by omission of a metabolic substrate is also in keeping with the opinion that [^{3}H]purine release is a consequence of an altered energy metabolism in the synaptosomes.

A recent study of White (1977) suggests that there may be in addition a direct release of ATP from synaptosomes following depolarization. He incubated a synaptosomal preparation with a luciferin luciferase enzyme mixture capable of producing chemiluminescence in the presence of ATP Potassium (28–51 mM) and veratridine (50 μM) produced an increased chemiluminescence corresponding to an increase in ATP concentration of approximately 5×10 M The response to potassium was at least partly calcium-dependent while that to veratridine was not Under our experimental conditions (10 μM veratridine) there was a good calcium dependence. These discrepancies could mean that we and White have studied different phenomena However the results of White (1977) suggest that some of the [H]purine release found in the present study could be due to the direct release of [H]ATP

However we found no clear evidence that any

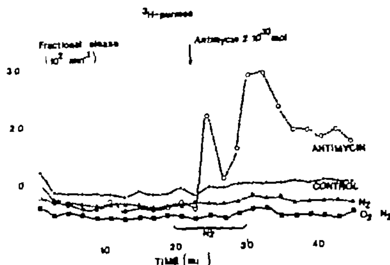


Fig. 4. The effect of antimycin (2 · 10^{-10} mol in 0.1 ml) and of reducing the buffer O_2-content (N_2-bubbling) on the release of [³H]purines from synaptosomal bed. One representative experiment with 4 parallel perfusions. ●—● Control; ○—○ antimycin administered after 24 min, ▲– –▲, buffer continuously gassed with 95% N_2, 5% CO_2, ■—■, buffer gassed with 95% O_2, 5% CO_2 during first 20 and last 15 min and with 95% N_2, 5% CO_2 in between.

...ficant amounts of adenine nucleotide were re...ed. It can of course be argued that the ...leotides released are very rapidly metabolized ...he compounds actually detected (adenosine, in...ne, hypoxanthine). However the present tech...ue using superfusion of a synaptosomal bed of ...all size would tend to minimize reuptake and ...tabolism. Furthermore we found that when the ...aptosomes were subjected to a hypo-osmolar ...ck there was a very significant release of ...cleotide material. This shows that nucleotides ...leased from such a preparation may be found as ...ch in the superfusate. Thus the present results ...gest that if intact ATP is released it forms but a ...inor part of the total release. However further ...dies are clearly necessary to evaluate the very ...portant question if ATP is released as such in the ...ntral nervous system which is a prerequisite for a ...nsmitter role similar to that it is suggested to play ...for example gut and urinary bladder (Burnstock ...77).

We found no significant effect of glutamate, ...stamine or acetylcholine on the [³H]purine release ...om hypothalamic synaptosomes. Pull & McIlwain ...975) found a small (30%) increase in purine re...ase from cortical slices by histamine and ...etylcholine in the concentrations used here. Release of this magnitude may have escaped our detection. On the other hand, these authors found a 6-fold increase in purine release following administration of glutamate. Had that occurred in the present experiments it would certainly have been detected. It is therefore necessary to assume that there is a difference between slices and a broken cell preparation in its responsiveness to glutamate. It is therefore interesting to note the selective toxicity of monosodium glutamate recently reported by Simson et al. (1977). These authors found that monosodium glutamate injected into the hypothalamus produced a clearcut destruction of cell bodies but had no effect on axons. It is at least possible that the adenosine release found repeatedly following administration of glutamate in slice preparations (cf. Pull & McIlwain, 1975, Newman & McIlwain 1977) requires the presence of intact cell bodies, which are obviously absent in the present type of preparation. Very high concentrations of noradrenaline was, however, found to enhance [³H]purine release from synaptosomes. Since this effect was not inhibited by either α- or β-adrenoceptor-antagonists the significance of the finding is dubious. It is interesting, that a noradrenaline activated Na⁺ K⁺-dependent ATPase has been detected in synaptosomes (Logan & O'Dono-

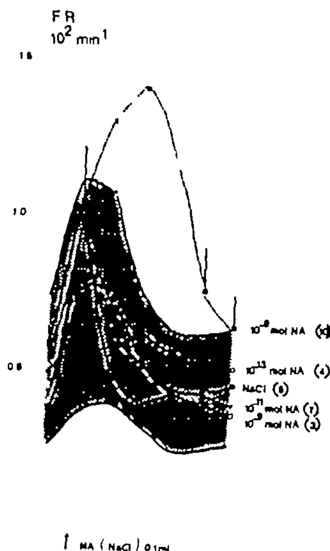


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van 1976) This enzyme may be related to catecholamine uptake rather than to a catecholamine receptor which could explain the absence of an effect of the receptor antagonists Furthermore the enzyme was inhibited by 1 mM EGTA (Logan & O Donovan 1976) as was the noradrenaline induced [^{3}H]purine release

In conclusion the present study has demonstrated the release of purines of which adenosine and its immediate breakdown products dominate from a crude rat hypothalamic synaptosome preparation The release is considerably enhanced by depolarization and by inhibition of energy metabolism (antimycin and omission of glucose) In view of the large number of effects of adenosine in the central and peripheral nervous system the finding may have some bearing physiological phenomena In particular it may be relevant that adenosine decreased neuronal firing rate (Phillis & Edstrom 1976 Siggins et al 1976 Kuroda et al 1976) and increased glycogenolysis (Wilkening & Makman 1976) The former effect may be expected to reduce energy utilization and the latter to increase substrate availability Thus the effects of adenosine are such as to reduce its formation

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)istensibility' of the papaverine-relaxed vascular bed ı human subcutaneous tissue

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HENRIKSEN O & KRISTENSEN J K.. Distensibility' of the papa erine-relaxed vascular bed in human subcutaneous tissue. Acta Physiol Scand 1979 106: 109–113 Received 7 July 1978. ISSN 0001-6772. Department of Nuclear Medicine and the Department of Dermatology Rigshospital, Copenhagen, Denmark.

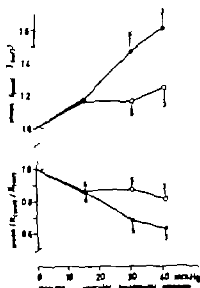
The effect of an increase in vascular transmural pressure upon the blood flow in two subcutaneous vascular beds, maximally dilated by papaverine was studied in 6 healthy humans. Blood flow was measured on the dorsum of the hand and at the lateral malleolus by the local ^{133}Xe washout technique. Increase in vascular transmural pressure was induced by lowering the labelled area various distances below heart level. Lowering the area caused an increase in blood flow The increase was less pronounced in the legs than in the hand. As arterial perfusion pressure head remained constant during lowering, this indicates that the relative decrease in ascular resistance was smaller in the leg than in the hand. Experimental edema did not influence the relative decrease in vascular resistance The results suggest that distensibility' of the resistance vessels is smaller in the leg than in the hand. This might be due to structural adaptation of the vascular wall in essels often subjected to increased hydrostatic pressure.

idence from animal and human studies indicates hat the increase in ascular resistance in response o nor-adrenaline is more pronounced in vessels ubjected to arterial hypertension (Folkow et al 1958, Conway 1963 Sivertsson 1970 Folkow et al. 1973 Hansen & Bohr 1975) The augmented increase in vasoconstriction might be due to adaptive structural changes of the ascular wall secondary to the increased ascular transmural pressure (Folkow et al. 1973 Folkow 1975)

Harting et al (1978) found that the increase in postischemic blood flow in the anterior tibial muscle during increase in vascular transmural pressure of 50 mmHg induced by external suction was less in patients with essential arterial hypertension than in normals.

Hallback et al (1974) studied the distensibility of ... e essels in spontaneously hypertensi e ... mpared with normotensive control rats. ... riments were pe rmed in alloperfused ... bility of resistance ... e changes in total ... rtly after a sudden change in blood flow The experiments were performed at different levels of vascular tone including atonia induced by papaverine. In the spontaneously hypertensive rats the relative change in ascular resistance following the change in blood flow and accompanying change in arterial perfusion pressure head was considerably smaller than that observed in the normotensive controls. These findings indicate a lower distensibility of the resistance vessels in the spontaneously hypertensive rats. This could be due to structural changes induced by the increased vascular transmural pressure.

In healthy humans, Paaske & Henriksen (1975) observed that the relative increase in vascular resistance during lowering the extremity was more pronounced in the leg than in the forearm. As the vessels in the legs are often subjected to an increased ascular transmural pressure, this indicates that vascular changes induced by increased transmural pressure are also present in normal subjects and are normal adaptive responses. Histological and chemical studies of the internal saphenous vein in man indicate that there is more



2. Mean relative blood flow (f_{low}/f_{ref}) and mean ...g vascular resistance (R_{low}/R_{ref}) obtained in arm ... leg plotted against increase in vascular transmural ...ure (mmHg). ● arm, ○ leg. Vertical lines with bars ...te 1 S.E.

...n example of the ^{133}Xe washout curves is shown ...ig. 1 and the results are shown graphically in ... 2. When the area was lowered 20 cm blood ... increased by 17% in both hand and leg, cor...ponding to a decrease in calculated total vascular ...istance of 14%. Lowering the area 40 cm caused ...ncrease in blood flow of 46% in the hand and ...% in the leg, corresponding to a decrease in total ...cular resistance of 32 and 13% in hand and ...g, respectively. The difference in response was ...nificant, $P<0.05$. When the area was lowered 60 ...n, blood flow increased by 60% in the hand and ...% in the leg, corresponding to a decrease in total ...scular resistance of 37 and 19% in hand and leg, ...spectively. This difference in response was sig...ficant, $P<0.05$.

Thus, when the area was lowered more than 20 ...n, the increase in blood flow was less pronounced ... the leg than in the hand.

...rterial perfusion pressure

...our expts. were carried out in 2 subjects. In all ...xpts. the increase in blood flow cessation pres...ure in arms and legs produced by lowering arms ...nd legs equalled the hydrostatic pressure exerted ...y a blood column of similar height.

Edema

Eight expts. were carried out in 2 subjects. In both subjects neither in hand nor in leg did the edema influence the observed relative increase in blood flow during lowering.

DISCUSSION

The ^{133}Xenon washout rates in the leg observed at reference level are greater than those observed by Levin Nielsen & Sejrsen (1972) during reactive hyperemia following 30 min of vascular occlusion. This indicates that paralysis of the vascular smooth muscle cells was achieved in the present expts. Both in hand and leg, blood flow increased during lowering. The increase in blood flow might be due to an increase in the arterial perfusion pressure head and/or a passive dilatation of the vascular bed.

Arterial perfusion pressure head

Under normal conditions, arterial and venous pressures increase in parallel during lowering. In a vascular bed in which the vessels act passively to changes in vascular transmural pressure arterial perfusion pressure head might increase due to vasoconstriction in surrounding vascular beds reacting normally to changes in vascular transmural pressure creating a redistribution of blood flow "inverse steal" (cf. Lassen & Westling 1969). However both in arm and leg the increase in blood flow cessation pressure in the area infiltrated with papaverine during lowering equalled hydrostatic pressure exerted by a blood column of a similar height. This indicates that in our experiments local arterial perfusion pressure head in a "passive vascular bed" is not affected by "inverse steal" but remains constant during lowering. The observed increase in blood flow during lowering is, therefore, presumably exclusively due to distension of vascular bed.

Distensibility of the vascular bed

The curves in Fig. 2 suggest that distensibility of the vascular bed is smaller in the leg than in the hand, when vascular transmural pressure is elevated more than 15 mmHg. As the in situ distensibility of the vascular bed depends on the distensibilities of the vascular and the extravascular tissue ($1/D = 1/D + 1/D$) the observed difference in distensibility of the vascular bed might be due to differences in vascular compliance and/or tissue compliance.

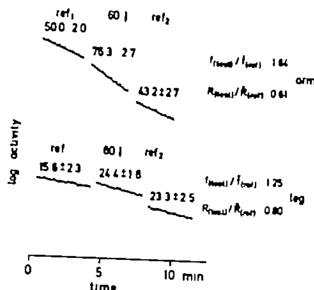


Fig 1 Example of ^{133}Xenon washout curves in human subcutaneous tissue infiltrated with papaverine obtained in arm and leg placed horizontally lowered 60 cm and finally placed horizontally Figures denote calculated washout rate constant times 10 10^3 (min) ± 1 S E

the media in the lower part than in the upper part of the vein (Kügelgen 1955 Svejcar et al 1962) As this difference in muscle content could not be found in children who had not yet begun to walk this indicates that in adults the increased hydrostatic pressure in the upright position might influence muscle tissue content The accentuated vasoconstriction in the leg might be due to structural changes of the arterioles as observed in the spontaneously hypertensive rats or due to an increased sensitivity of the vascular smooth muscle cells to nor adrenaline (Hansen & Bohr 1975)

If these changes in the vessels are structural they should be expected to be accompanied by a decrease in distensibility of the vessels when their smooth muscles are completely relaxed Therefore we studied the effect of an increase in vascular transmural pressure upon the resistance in subcutaneous vessels in the arm and leg of normal humans under conditions of vascular relaxation induced by papaverine

METHODS

The investigations were carried out in 6 healthy humans placed in a supine position Blood flow in subcutaneous tissue was estimated by the local ^{133}Xe washout technique (Sejrsen 1971)

^{133}Xe in 0.3 ml isotonic saline mixed with papaverine (8 mg) in order to paralyse the vascular smooth muscle cells was injected s c. on the dorsum of the hand and t the lateral malleolus Increase in vascular transmural pressure was induced by lowering the area various distances ... ing maximal lowering below the jugular notch. The extremity was carefully immobilized in order to avoid changes in counting geometry

A single experiment consisted of a triad of measurements. (1) the area under study placed at jugular level k_{ref_1}, ( ) lowered, k_{lowe}, and finally (3) placed at jugular level k_{ref_2} Each period of measurement lasted about 1 min.

Arterial perfusion pressure

Changes in local arterial perfusion pressure in a maximally dilated vascular bed during lowering was studied in ... subjects Four experiments were carried out. Blood flow was measured on the distal part of the forearm and just proximal to the lateral malleolus. Pressure as exerted locally upon the skin by inflating a cuff placed corresponding to the area under study The cuff-pressure was successively increased by 10 mmHg starting from 70 mmHg Each period of measurement lasted about 6 min Local arterial perfusion pressure was estimated as the minimal pressure to be exerted in order to stop the washout of ^{133}Xe blood flow cessation pressure.

Edema in subcutaneous tissue

The influence of edema formation upon relative blood flow in the maximally dilated vascular bed lowering was studied in subjects Eight experiments were performed Edema was produced by local suction of about 100 mmHg for 30 min The edema produced was very pronounced as the thickness of a skinfold between 2 selected points of the skin increased by about 300 %

Calculations and statistics

Mean perfusion coefficient f can be calculated from the Kety-equation.

$f = k \cdot \lambda \cdot 100$ (ml 100 g min) (Kety ...)

where k denotes the washout rate constant in min^{-1} and the subcutaneous tissue to blood partition coefficient ml/g.

Relative blood flow during lowering $f_{lowe}/\bar{f}_{ref}$ ... calculated as $k_{lowe}/\bar{k}_{ref}$, since λ was assumed to remain constant during a triad of measurements. $\bar{k}_{ref}$ and $\bar{f}_{ref}$ denote the average of the washout rate constants and perfusion coefficients respectively obtained just before and after lowering.

The k_{lowe} and $\bar{k}_{ref}$ values were compared by Student *t* test for paired samples The calculated relative blood flow values for the arm were compared with those for the leg by the randomization test for paired samples. Limit of significance was chosen as 0 05

RESULTS

At reference level the following washout rate constants (mean ±1 S E) were obtained. Hand 0 049±0 006 min leg. 0 038±0 005 min (n = 21)

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Edema formation might change tissue compliance (Guyton 1965) and total cross-sectional area of a passive vascular bed (Zelis Lee & Mason 1974) Edema produced by local suction of about −100 mmHg for 30 min was very pronounced as the thickness of a skinfold between two points increased by 300% and probably decreased interstitial compliance (Guyton 1965) As the induced edema will cause a decrease in λ it is not possible to deduce the effect of edema upon reference blood flow However by comparing the washout rate obtained during lowering with the average value of the washout rates obtained at reference level just before and after the test errors due to gradual changes in λ tend to be eliminated This indicates that the calculated relative blood flow during lowering is not significantly influenced by this error Neither in hand nor in leg did the edema influence the response in blood flow to lowering indicating that interstitial compliance even when it is decreased by marked edema does not significantly limit distension of the passive vascular bed in subcutaneous tissue in response to increase in vascular transmural pressure This is compatible with the observation that there was no difference in the increase in blood flow during lowering in the papaverine induced passive vascular bed in subcutaneous tissue in patients suffering from generalized scleroderma as compared to normal controls (Kristensen & Henriksen 1977) The results suggest that the observed difference in distensibility of the vascular bed in hand and leg is not due to differences in distensibility of surrounding structures but to differences in distensibility of the vascular walls

The method used in the present study gives only rough information about distensibility of the *total* vascular bed This is probably the reason why there was no difference in relative blood flow in leg and arm during lowering corresponding to an increase in vascular transmural pressure of about 15 mmHg The observed decrease in total vascular resistance mostly reflect a decrease in resistance in precapillary section of the vascular bed because even in the maximally dilated vascular bed about 60% of the total vascular resistance is located in the precapillary section Furthermore during lowering the precapillary part of the vascular bed will account for a greater part of total vascular resistance as these vessels presumably are considerably less distensible than the vessels in the postcapillary section of the vascular bed Therefore the observed difference in distensibility of the vascular bed in [illegible] and leg is presumably mostly due to differences [illegible] distensibility of the vessels in the precapillary [illegible]tion of the vascular bed This is in agreement [illegible] the observations in spontaneously hypertensive [illegible] (Hallbäck Lundgren & Weiss 1962)

Thus the results suggest that structural adaptation of the vascular wall subjected to prolonged increase in vascular transmural pressure (Folkow [illegible] al 1973 Folkow 1975) is present in normal subjects The augmented vasoconstrictor response [illegible] relatively greater increase in resistance in response to increase in vascular transmural pressure [illegible]served in the leg of healthy humans (Paaske & Henriksen 1975) is therefore most likely due to a [illegible]mal adaptive structural change in the walls of [illegible] arterioles in response to prolonged exposure [illegible] high transmural pressures

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egional blood flow in canine myocardium as determined y local washout of a freely diffusible radioactive ıdicator

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HAUNSØ S AMTORP O & LARSEN B Regional blood flow in canine myocardium as determined by local washout of a freely diffusible radioactiv indicator Acta Physiol Scand 1979 106: 115–121 Received 7 Aug. 1978. ISSN 0001-6772. Department of Cardiology Copenhagen County Hospital, Gentofte Denmark.

The aim of this study has been to examine *the utility of the washout of freely diffusible* radioactive indicator as a measure of regional myocardial blood flow in open-chest anesthetized dogs. The method employed was direct intramyocardial injection of Xenon-133 followed by measurement of its gamma radiation. The experimental washout curves show after a short acceleration period, monoexponential washout of the indicator over two decades. W found indications of insignificant veno-arterial shunting by diffusion of the blood flow level measured, insignificant arterial recirculation of the indicator and minimal radioactive contribution to precordial residue versus time curves arising from right heart or non-myocardial tissue We suggest that diffusion equilibrium between the tissue in the counting field and the blood leaving it is maintained during the linear down slope of two decades of the clearance curve, and that local blood flow can be calculated from washout rate constant obtained from this part of the curve. The method employing intramyocardial injection of Xenon-133 was found to give the same results as atraumatic epicardial labelling indicating negligible effect of the injection trauma and supporting the validity of the local injection method using small volumes (? 10 µl)

Key words Intramyocardial application, atraumatic application

The Kety-Schmidt method (1945) for measurement of blood flow was applied to the heart by Herd et al. (196.) and reinvestigated by Ross et al. (1964) Johansson et al (1964) and Doutheil & Rohde (1966) The radioactive gases Krypton-85 or Xenon-133 ere injected into the coronary artery and the disappearance of the indicator from the myocardium as recorded by precordial counting. By assuming a single homogeneous tissue with steady flow and diffusion equilibrium between the tissue in the counting field and the blood leaving *it*, capillary blood flow can be expressed per 100 g of tissue in accordance with the term:

$$f \; k \; \lambda \; 100 \,(\text{ml}/100\,\text{g} \;\; \text{min})$$

(Kety & Schmidt (1945) Kety (1951)) where k is the rate constant measured from the washout curve and λ is the partition coefficient between the tissue and blood. If various parts of the tissue have different *f*/λ values, this method is inapplicable (the slope of the washout curve might only pro ide a rough measure of the mean values of *f*)

By use of local *deposition of the indicator it is* possible to measure clearance of the isotope in particular areas of the exposed myocardium (Lindner 1966, *Sullivan et al* 1967 *Brandi et al.* 1968, Andersen et al. 1969) However inter-tissue diffusion *combined with* absorption of beta radiation might invalidate the use of Krypton-85 for myocardial blood flow measurements as it does in measurements of skin blood flow (Sejrsen 1967)

Using local injections of Xenon-133 for blood flow measurements, considerations about the effect of tissue trauma, the homogeneity of blood flow and the uniformity of tissue labelling (Lassen 1967) must

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By use of local deposition of the indicator it is possible to measure clearance of the isotope in particular areas of the exposed myocardium (Lindner 1966 Sullivan et al. 1967 Brandi et al 1968, Andersen et al. 1969). However inter-tissue diffusion combined with absorption of beta radiation might invalidate the use of Krypton-85 for myocardial blood flow measurements as it does in measurements of skin blood flow (Sejrsen 1967)

Using local injections of Xenon-133 for blood flow measurements, considerations about the effect of tissue trauma, the homogeneity of blood flow and the uniformity of tissue labelling (Lassen 1967) must

be made Local injection of a small volume of indicator solution into skeletal muscle initiates a hyperemia which cannot be avoided by a reduction of the injected volume (Tonnesen & Sejrsen 1970) However this problem has been solved for some tissues by introduction of the atraumatic indicator application (Sejrsen 1968 Sejrsen & Tonnesen 1968) In the present report the local injection method has been evaluated partly by comparing myocardial blood flows obtained after traumatic and atraumatic introductions of the indicator into the same area and partly by analysis of the washout curves obtained after local injections into different depths of the myocardial wall by repeated injections into the same area and by injections of various volumes of indicator solution

METHODS

Nine mongrel dogs of either sex weighing between 19 and 30 kg were used The dogs were premedicated by subcutaneous injections of 2 propyl-10(dimethylaminopropyl)ftiazin (Combelene®) Anesthesia was introduced by thiomebumal sodium 12.5 mg/kg i v An endotracheal tube was introduced and artificial respiration with oxygen and nitrous oxide in the volume ratio 1 to 3 was maintained with a respirator After 10 mg droperidolum (Dehydrobenzperidol®) i.v injections of fentanyl (Haldid®) 0 1 mg i v and pancuronibromidum (Pavulon®) 2 mg i v were given when required

General examination schedule

A pigtail" catheter was introduced through the left femoral artery and the tip was placed in the ascending aorta A Swan-Ganz catheter was inserted through the right external jugular vein with the tip placed in the pulmonary artery Aortic and pulmonary arterial blood pressures were recorded continuously by means of a Statham pressure transducer (model P23Db) situated at heart level and together with the ECG visualized on a UV recorder (model 5656) ABEM Body temperature was maintained by external heating and controlled by a thermistor in the rectum Arterial oxygen saturation oxygen and carbon dioxide tensions, pH and hematocrit were recurrently measured The heart was exposed through a left thoracotomy in the fifth intercostal space and a slit was made in the pericardium. To prevent atelectasis after thoracotomy a positive end-expiratory pressure of 3 cmH_2O was maintained through the entire procedure The epicardium was kept moistened at a constant temperature (38°C) by heated sodium chloride solution (0 9%) After the preparation the animals were allowed to stabilize as indicated by normal arterial blood pressures and gas tensions

Methods of tracer application

Xenon-133 (10 mCi/ml) dissolved in sterile saline (obtained from the Radiochemical Centre Amersham England) was introduced into myocardial tissue by two different routes. (1) Isotope solution in amounts of 5 [illegible] µl was injected directly into the myocardium of left ventricle The injections were performed with a needle [illegible] mm o d.) inserted perpendicularly to the epicardial surface A moveable collar on the needle determined [illegible] depth of the local injection and hence the position of the tracer depot. (2) Through a slit in the pericardium a [illegible] membrane (2×3 cm) 20 µm thick was inserted between peri- and epicardium of the left ventricle covering [illegible] between two anterior ventricular branches of the left descending coronary artery The pericardium ensured a stable position of the membrane which remained firmly attached to the epicardial surface. With an angled needle [illegible] µl of isotope solution was carefully placed under the membrane near the center to obtain uniform distribution over an area of the epicardium.

The following experiments were performed

(*a*) Five dogs were used to compare myocardial blood flows determined after traumatic injections at a depth of [illegible] mm and atraumatic applications of the indicator on the same area of the exposed myocardium. In one of the dogs the two methods were compared during low flows obtained by graded bleeding to stable arterial blood pressure levels of 80 60 and 40 mmHg. Paired values were obtained by injecting Xenon-133 through the mylar membrane [illegible] the myocardium as soon as the washout curve after epicardial atraumatic labelling had reached background activity In one of the dogs mentioned the heart was arrested and radioactivity of Xenon-133 versus time was measured following atraumatic and traumatic indicator application in the corresponding epicardial area. During this experiment the distance between the scintillation detector and the exposed heart was reduced from 4[illegible] cm (*b*) In dogs washout curves following superficial intramyocardial injections of Xenon-133 at a depth of [illegible] mm were compared to washout curves following mid-myocardial injections (depth 5 mm). Paired values were obtained by injecting Xenon-133 mid-myocardially as soon as the washout curve after superficial injection had reached background activity (*c*) In one dog a series of [illegible] mid-myocardial injections (5 µl) were performed into the same epicardial spot Each injection was made immediately following the preceding washout. (*d*) In [illegible] additional dog, washout curves were obtained after mid-myocardial injections (depth 5 mm) using a volume of [illegible] or 10 µl of indicator solution The thickness of the ventricular wall at the sites of injections was about 11 mm

Recording of gamma radiation

The gamma radiation of Xenon-133 was monitored by a scintillation detector with a thallium activated sodium iodide crystal placed 7 cm inside the opening of a [illegible] collimator The collimator was positioned at a distance of 14 cm above the anterior wall of the exposed left ventricle in order to obtain count rates from the whole heart. The position of the detector was kept constant during the entire course of each experiment (except in one expt) [illegible] using an amplifier/analyzer (Meditronic Denmark) the gamma emission around the 81 KeV peak of Xenon-133 was recorded. The crystal was connected to a [illegible]

[Ta]ble 1 Blood gas and hemodynamic parameters of nine dogs measured before and recurrently during [eac]h [e]xperiment

[Th]e figures are mean values ±S.D *N* number of observations

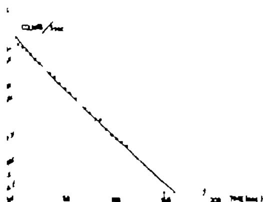
[...] ([m]mHg)	$PaCO_2$ (mmHg)	pH	Hct.	Heart rate (beat min⁻¹)	Aortic pressure (mean) (mmHg)	Pulmonary pressure (mean) (mmHg)
[...]±28 N=27	27±5 N=27	7.43±0.05 N=26	33±4 N=27	139±16 N=27	111±17 N=27	11±3.0 N=27

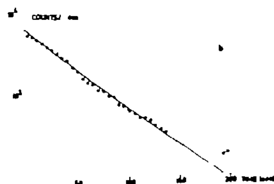
[...]ster. The counting of radioactivity began immediate[ly afte]r each local indicator application. The counts were [acc]umulated over periods of 5 s, corrected for background [activi]ty and plotted in semilogarithmic diagrams. The [biolo]gical half-time (t½) was determined by "fitting" [a strai]ght line by eye to the recorded points. The effect of [radiat]ion arising from other parts than the region of in[terest] was examined by placing a lead sheet (2 mm thick) [with] bore holes (1.0 or 3.0 cm) close to the heart during [cou]nting following mid-myocardial injection and epicar[dial] labelling.

[RE]SULTS

[Ta]ble 1 shows the hemodynamic parameters, res[pira]tory parameters and hematocrit values ob[tain]ed in the nine dogs included in the study [(ex]cluded are only values obtained during graded [ble]eding). As the partition coefficient is dependent [on] the hematocrit, values corresponding to meas[ure]d hematocrit values (Tonnesen & Sejrsen 1967) [we]re used for calculation of blood flows.

Fig. 1 shows semilogarithmic plots of representative washout curves recorded (*a*) after direct intramyocardial injection (5 mm) and (*b*) after atraumatic epicardial labelling obtained subsequently from overlying epicardial area and without additional collimation. The curves have virtually the same shape with a smaller slope initially followed by fairly linear downslopes. When collimation limited the detection to the region of injection, the washout curve fitted a straight line from peak count rate through two decades in a semilogarithmic plot as shown in Fig. 2. The tail-part of the curve is diminished by collimation. No significant difference was found between measurements of blood flow obtained with and without close collimation. Fig. 3 illustrates two washout curves detected with collimation limited to the corresponding region of epicardial application and mid-myocardial injection of the indicator. As demonstrated no significant difference was found between the linear downslopes of the curves from peak count to back ground level.





[Fi]g. 1. Two semilogarithmic plots of Xenon-133 washout [cu]rves recorded over the exposed heart. (*a*) Curve [ob]tained immediately after intramyocardial injection of 5 [µl] indicator solution. (*k* 1.49 min⁻¹) (*b*) Curve obtained immediately after subsequent application of 4 µl indicator solution under mylar membrane covering the epicardium. (*k* = 1.34 min⁻¹)

be made Local injection of a small volume of indicator solution into skeletal muscle initiates a hyperemia which cannot be avoided by a reduction of the injected volume (Tonnesen & Sejrsen 1970) However this problem has been solved for some tissues by introduction of the atraumatic indicator application (Sejrsen 1968 Sejrsen & Tonnesen 1968) In the present report the local injection method has been evaluated partly by comparing myocardial blood flows obtained after traumatic and atraumatic introductions of the indicator into the same area and partly by analysis of the washout curves obtained after local injections into different depths of the myocardial wall by repeated injections into the same area and by injections of various volumes of indicator solution

METHODS

Nine mongrel dogs of either sex weighing between 19 and 30 kg were used The dogs were premedicated by subcutaneous injections of propyl-10(dimethylaminopropyl)flutiazin (Combelene®) Anesthesia was introduced by thiomebumal sodium 12.5 mg/kg i v An endotracheal tube was introduced and artificial respiration with oxygen and nitrous oxide in the volume ratio 1 to 3 was maintained with a respirator After 10 mg droperidolum (Dehydrobenzperidol®) i v injections of fentanyl (Haldid®) 0 1 mg i v and pancuronibromidum (Pavulon®) 2 mg i v were given when required

General examination schedule

A pigtail" catheter was introduced through the left femoral artery and the tip was placed in the ascending aorta A Swan-Ganz catheter was inserted through the right external jugular vein with the tip placed in the pulmonary artery Aortic and pulmonary arterial blood pressures were recorded continuously by means of a Statham pressure transducer (model P23Db) situated at heart level and together with the ECG visualized on a UV recorder (model 5656) ABEM Body temperature was maintained by external heating and controlled by a thermistor in the rectum Arterial oxygen saturation oxygen and carbon dioxide tensions pH and hematocrit were recurrently measured The heart was exposed through a left thoracotomy in the fifth intercostal space and a slit was made in the pericardium. To prevent atelectasis after thoracotomy a positive end-expiratory pressure of 3 cmH_2O was maintained through the entire procedure The epicardium was kept moistened at a constant temperature (38°C) by heated sodium chloride solution (0.9%) After the preparation the animals were allowed to stabilize as indicated by normal arterial blood pressures and gas tensions.

Methods of tracer application

Xenon-133 (10 mCi/ml) dissolved in sterile saline (obtained from the Radiochemical Centre Amersham England) was introduced into myocardial tissue by [illegible] ferent routes. (1) Isotope solution in amounts of 5 [illegible] µl was injected directly into the myocardium of the left ventricle The injections were performed with a needle [illegible] mm o.d) inserted perpendicularly to the epicardial surface A moveable collar on the needle determined the depth of the local injection and hence the position of the tracer depot (2) Through a slit in the pericardium a [illegible] membrane (2×3 cm) 70 µm thick was inserted between peri and epicardium of the left ventricle covering an area between two anterior ventricular branches of the left descending coronary artery The pericardium ensured a stable position of the membrane which remained firmly attached to the epicardial surface With an angled needle [illegible] µl of isotope solution was carefully placed under the membrane near the center to obtain uniform distribution over an area of the epicardium

The following experiments were performed

(*a*) Five dogs were used to compare myocardial blood flows determined after traumatic injections at a depth of [illegible] mm and atraumatic applications of the indicator in the same area of the exposed myocardium. In one of the dogs the two methods were compared during low flows obtained by graded bleeding to stable arterial blood pressure levels of 80 60 and 40 mmHg Paired values were obtained by injecting Xenon-133 through the mylar membrane [illegible] the myocardium as soon as the washout curve after epicardial atraumatic labelling had reached background activity In one of the dogs mentioned the heart was arrested and radioactivity of Xenon-133 versus time was measured following atraumatic and traumatic indicator application in the corresponding epicardial area. During this experiment the distance between the scintillation detector and the exposed heart was reduced from 14 [illegible] cm. (*b*) In dogs washout curves following superficial intramyocardial injections of Xenon-133 at a depth of [illegible] mm were compared to washout curves following mid-myocardial injections (depth 5 mm) Paired values were obtained by injecting Xenon-133 mid-myocardially as soon as the washout curve after superficial injection had reached background activity (*c*) In one dog a series of mid myocardial injections (5 µl) were performed in [illegible] the same epicardial spot. Each injection was made immediately following the preceding washout. (*d*) In an additional dog, washout curves were obtained after mid-myocardial injections (depth 5 mm) using a volume of [illegible] or 10 µl of indicator solution The thickness of the ventricular wall at the sites of injections was about 11 mm

Recording of gamma radiation

The gamma radiation of Xenon-133 was monitored by a scintillation detector with a thallium activated sodium iodide crystal placed 7 cm inside the opening of a [illegible] collimator The collimator was positioned at a distance of 14 cm above the anterior wall of the exposed left ventricle In order to obtain count rates from the whole heart. The position of the detector was kept constant during the entire course of each experiment (except in one exp [illegible]) using an amplifier/analyzer (Meditronic Denmark) the gamma emission around the 81 keV peak of Xenon-133 was recorded The crystal was connected to a

…le 1 Blood gas and hemodynamic parameters of nine dogs measured before and recurrently during …h experiment

figures are mean values ±S.D., N number of observations

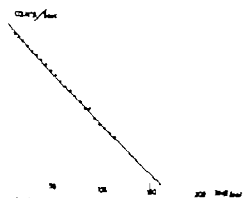
…Hg)	P_aCO_2 (mmHg)	pH	Hct.	Heart rate (beat min⁻¹)	Aortic pressure (mean) (mmHg)	Pulmonary pressure (mean) (mmHg)
±30 17	27±5 N 27	7.43+0.05 N 26	33±4 N 27	139±16 N 27	111±17 N 27	11+3.6 N=27

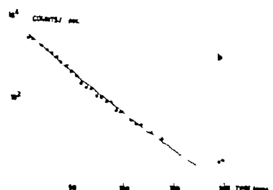
…nder. The counting of radioactivity began immediate… …ter each local indicator application. The counts were …mulated over periods of 5 s, corrected for background …ty and plotted in semilogarithmic diagrams. The …pical half-time (t½) was determined by fitting …ght line by eye to the recorded points. The effect of …tion arising from other parts than the region of in… …t was examined by placing lead sheet (2 mm thick) …bore holes (1.0 or 3.0 cm) close to the heart during …out following mid-myocardial injection and epicar… labelling.

…SULTS

…le 1 shows the hemodynamic parameters, res…atory parameters and hematocrit values ob…ed in the nine dogs included in the study …cluded are only values obtained during graded …ding). As the partition coefficient is dependent …the hematocrit, values corresponding to mea…d hematocrit values (Tønnesen & Sejrsen 1967) …e used for calculation of blood flows.

…g. 1 shows semilogarithmic plots of representa-tive washout curves recorded (*a*) after direct intramyocardial injection (5 mm) and (*b*) after atraumatic epicardial labelling obtained subsequently from overlying epicardial area and without additional collimation. The curves have virtually the same shape, with a smaller slope initially followed by fairly linear downslopes. When collimation limited the detection to the region of injection, the washout curve fitted a straight line from peak count rate through two decades in a semilogarithmic plot as shown in Fig. … The tail-part of the curve is diminished by collimation. No significant difference was found between measurements of blood flow obtained with and without close collimation. Fig. 3 illustrates two washout curves detected with collimation limited to the corresponding region of epicardial application and mid-myocardial injection of the indicator. As demonstrated no significant difference was found between the linear downslopes of the curves from peak count to background level.





…1. Two semilogarithmic plots of Xenon-133 washout …ves recorded over the exposed heart. ( ) Curve …ained immediately after intramyocardial injection of 5 … indicator solution. (k 1.49 min⁻¹) (*b*) Curve obtained immediately after subsequent application of 4 µl indicator solution under mylar membrane covering the epicardium. (k=1.34 min⁻¹)

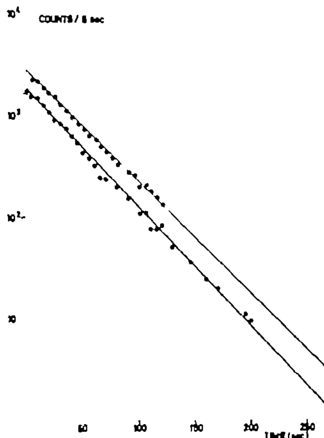


Fig 2 Semilogarithmic plots of Xenon-133 washout obtained by registration from the whole heart (solid circles) and by registration limited by collimation to the region of injection of 5 μl indicator solution at depth 5 mm (open circles)

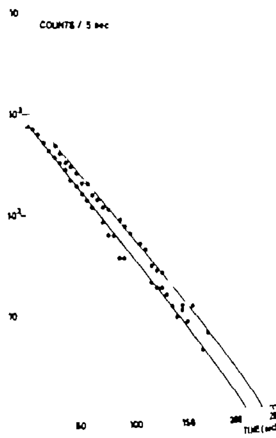


Fig 3 Semilogarithmic plots of Xenon-133 washout obtained by registration limited by collimation to the corresponding region after epicardial application of 4 μl indicator (open circles) and mid-myocardial injection of 5 μl indicator (solid circles)

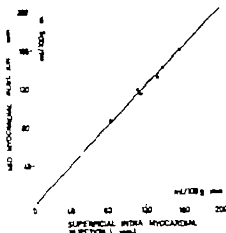
Blood flow calculated from clearance rates in 5 dogs (group a) following local mid-myocardial injections ($n=23$) was 178 0±34 3 ml/100 g min and following epicardial application of Xenon-133 was ($n=18$) 134 4±42 6 ml/100 g min (means±S D) Using Student s t test for unpaired samples with a significance level of 0 05 the difference was not statistically significant

The residue versus time curves in a non beating heart after epicardial labelling and subsequent local mid-myocardial injection in the corresponding epicardial area were almost horizontal indicating that loss of tracer by diffusion accounts for only a negligible part of the fall of the experimental washout curves Changing the distance of the detector did not change the slope of the curve

Fig 4 shows a comparison between myocardial blood flows calculated from curves obtained after superficial intramyocardial (depth 1 mm) and mid-myocardial (depth 5 mm) injections of 5 μl indicator solution (group b) The coefficient of correlation r is 0 96 The slope of the regression line b is 1 1 and the y-intercept −14 1 The standard deviation of intercept SD_a is 12 1 It is assumed that the true line passes through 0 0 The slope of this line calculated according to the formula $b=\Sigma xy/\Sigma x^2$ was 0 99 ($SD_b=0$ 02) which indicates that the slope is not significantly different from unity

Fig 5 shows a comparison between myocardial blood flows calculated from curves obtained following epicardial labelling and subsequent local mid-myocardial injection of Xenon 133 (group a). The coefficient of correlation r is 0 99 The slope of the regression line b is 0 80 and intercept 18 3 The standard deviation of y intercept SD_a is 5 8 Paired values in individual experiments were not statistically different ($P>0$ 05) using Student s t test for paired samples.

Washout curves following local mid-myocardial injections of Xenon-133 dissolved in fluid volume



4 Comparison between blood flows calculated from [illegible] curves following superficial intramyocardial [illegible] and subsequent mid-myocardial (ordinate) in[illegible] of Xenon-133

.. 5 or 10 µl (group *d*) were similar in respect to [illegible] shape and slope of the curves. Counting rates [illegible]owing injection of 5 µl of Xenon-133 solution [illegible] initial recordings above 6×10^4 counts per min[illegible], and were chosen as the best compromise be[illegible]een achieving adequate counting rates and keep[illegible] the injected volume small. In order to evaluate a [illegible]sible influence of local tissue trauma on the [illegible]asurement of blood flow a total of 10 such in[illegible]myocardial injections through the same epicar[illegible]al site were made, each immediately following the [illegible]shout of the preceding (group *c*). No difference [illegible]uld be observed between the shape of the curves, [illegible]dicating that local oedema or tissue damage did [illegible]t significantly influence myocardial blood flow [illegible]sing this technique

[D]ISCUSSION

[T]he generally accepted concept of flow limited [w]ashout of a freely diffusible indicator was de[sc]ribed by Kety & Schmidt (1945) and has been [a]pplied to various tissues (Lassen 1967). The basic [as]sumption is that washout of indicator from the [t]issue is limited solely by the convective transport [b]y blood flow

A monoexponential course of the total indicator washout curve suggests that almost complete [e]quilibrium is achieved between tissue and blood during the washout process. If this was not the case, the curve would depart from the linear course in a semilogarithmic diagram corresponding to a series of diffusion resistances of varying magnitude (Larsen & Lassen 1967) Kety (1951) did not consider the possibility that the indicator could exchange as shunting by diffusion between arterial and [v]enous blood allowing counter current exchange After local labelling of a small myocardial area with Xenon-133 veno-arterial shunting may occur since inflowing arteries and effluent veins are running concomitantly in the myocardium (Bassingthwaighte et al. 1974) If such a diffusional shunting is significant, then the measured flow value from the washout of a diffusible indicator will be underestimated if blood flow is calculated from the later part of the curve (Bassingthwaighte 1977)

In the present studies the washout curves were dominated by a monoexponential washout As seen in Fig. the washout curve fits a straight line from *peak count rate through two decades in a* semilogarithmic plot. It is likely that the removal of the indicator from the intramyocardial depot initially is somewhat lower due to an increase in the interstitial fluid content with reduced capillary density thus explaining the smaller slope as seen very early in the washout curve. As the injected fluid gradually becomes distributed into surrounding tissues the conditions for washout will improve with an acceleration of the rate of washout.

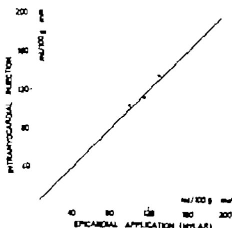


Fig 5 Comparison between myocardial blood flows calculated from washout curves obtained following epicardial (abscissa) and intramyocardial (ordinate) applications of Xenon-133 The line on the figure is y=x.

Arterial recirculation to myocardial tissue does not interfere with the estimation of the initial down slope of the curve because radioactive material leaving the myocardium enters right heart chambers from the coronary sinus and cardiac veins and passes to the lungs where 95% is removed during the first passage (Herd et al 1962) The recirculating 5% will be distributed in accordance with cardiac output and only a very small fraction will reach the counting field as recirculation Radioactivity from Xenon 133 in right heart chambers lungs or other non myocardial tissues within the field of the detector might nevertheless influence the acceleration period as seen in the initial part of the washout curve or may result in a prolongation of the downslope of the washout curves The problem was investigated by shielding the detector from radiation arising elsewhere than the region of injection When collimation limited the observation to this regional area monoexponential washout to background level was observed The slope of the washout curve did not differ significantly from that obtained by detecting with wide collimation except that the monoexponential fall persisted for a longer time Therefore radioactivity arising from right heart or non myocardial tissues makes no significant contribution to precordial residue versus time curves within the time corresponding to the initial downslope of one decade Furthermore veno-arterial shunting of Xenon-133 has hardly led to significant underestimates in the flows calculated in the present study since it has been shown by Sejrsen & Tønnesen (1972) to be an insignificant source of error in skeletal muscle in the range of f values encountered in the myocardium This finding is in accordance with the results of Bassingthwaighte (1968) and Bassingthwaighte & Yipintsoi (1970) who found indications of shunting by diffusion of Xenon 133 and other indicators but only when the coronary blood flow was relatively low The result that the slower tailpart of the washout curve found by detecting with wide collimation could be eliminated by detecting with close collimation can be interpreted as uptake of Xenon takes place in the fatty tissue around the large veins The basic assumption of complete equilibrium between tissue and blood by diffusion seems almost achieved and maintained during the monoexponential washout and therefore local blood flow can be calculated from the washout rate constant

Following atraumatic epicardial labelling with Xenon-133 the washout curve initially also exhib[illegible] an acceleration period This phenomenon is [illegible] doubtedly due to uptake by diffusion of Xenon [illegible] and water from the epicardial depot into the inter[illegible] tial space in the superficial layers of the myo[illegible] dium With gradual distribution into underlying [illegible] sue washout improves the fractional washout [illegible] increases and a fairly straight downslope over [illegible] decades appears when close collimation is [illegible] (Fig. 3) The tailpart of the curve observed [illegible] wide collimation may be due to uptake into [illegible] tissue at the epicardium and around large coron[illegible] vessels in which Xenon is highly soluble

As demonstrated no difference in the in[illegible] course of washout and calculated blood flow [illegible] superficial intramyocardial and mid-myocard[illegible] levels could be detected This result is in accord[illegible] ance with that obtained by counting the activity [illegible] tissue blocks of the left ventricular free wall [illegible] mediately after a bolus injection of Xenon [illegible] (Bagger 1977) The difference observed by Kirk [illegible] Honig (1964) in normal hearts may arise from [illegible] use of I 131 which is limited by diffusion across [illegible] capillary wall at normal blood flows (Lassen [illegible] and not only by the convective blood flow [illegible] difference reported by Brandi et al (1968) may [illegible] due to the much bigger volume injected (0.1-0 [illegible] causing a pronounced traumatic reaction and po[illegible] bly interfering to different extents with the per[illegible] sion of various regions

The theoretical requirements for calculation [illegible] blood flow from the slopes of Xenon 133 wash[illegible] curves from myocardial tissue seem to be satisf[illegible] However traumatic injection of the indicator [illegible] solved in a fluid volume could possibly influen[illegible] the rate of washout over a longer period by [illegible] hyperemic response lasting considerably long[illegible] than the local effect of the tissue dilution caused [illegible] the injected volume By epicardial application of [illegible] indicator it was possible to carry out an atraumat[illegible] labelling of superficial myocardial tissue No sig[illegible] nificant differences were found over a wide range [illegible] flow between the flow values obtained by [illegible] method and those obtained by the local inject[illegible] method pointing at a minimum traumatic effect [illegible] the injection The intramyocardial injection of Xe[illegible] non 133 is much easier to carry out than the epica[illegible] dial indicator application and thus seems to be [illegible] suitable method for further investigations concern[illegible] ing regional blood flow during variable physiologi[illegible] cal conditions

…orted by the Danish Heart Foundation.
…sh to thank Dr Per Sejrsen for inspiring discussion … criticism of our study. We also wish to thank Miss …tte Holm and Mr Morten Kjærumgård for excellent …ical assistance.

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asurements of cardiac output and organ blood flow ats using $^{99}Tc^{m}$ labelled microspheres

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HAFSTRÖM L., PERSSON B & SUNDQVIST K.. Measurements of cardiac output and organ blood flow in rats using $^{99}Tc^{m}$ labelled microspheres. Acta Physiol Scand 1979 106. 123–128. Received 14 Sept. 1978, ISSN 0001-6772. Department of Surgery and Department of Radiation Physics, Uni ersity of Lund, S eden.

The cardiac output and regional blood flow ha e been simultaneously determined in the anesthetized rat by using the reference organ method. $^{99}Tc^{m}$ labelled dextran 15 µm microspheres were injected in the left entricle while simultaneously an arterial reference sample was draw n at constant know rate. The values calculated regarding cardiac output agree well with previously recorded series. Regional organ blood flow in the rat is presented as ml min g tissue and values do agree with those reported by others. The results indicate that the reference organ method can be used in the rat for the study of cardiac output and regional blood flow

Key ords. Microspheres, ^{99}Technetium^{m} blood flow cardiac output

ods for cardiac output determination and or blood flow measurements are to a great extent d upon dye dilution techniques. The basic work is field was done by Stewart (1897) and Hamilt al. (1932) Kety & Schmidt (1948) used ni s oxide in their basic work on the dilution prin-

The theoretical background was defined by ier (1962, 1965) The introduction of radioactive ators was a most important improvement of procedure according to Bassingthwaighte s re- (1976). The radioactive indicators can be in- uced in the vascular system and studied on the s of the degree of average dilution. Tracers ering to the extravascular space—inert gases ^{133}Xe, ^{85}Kr)—can be used and studied on the s of wash-out or wash-in techniques (Lassen et 1961 Darle 1970) A further improvement in suring blood flow by tracer technique came n labelled microspheres were introduced by olph & Heymann in 1967) The theoreti- considerations for the use of microspheres in suring blood flow of organs have been dis- sed extensively (Wagner et al 1969 Hoffbrand orsyth 1969 Buckberg et al. 1971 Bartrum et al. 4). This technique was used for measuring car output as reported by Archie et al. (1973) and Bartrum et al. (1974) An arterial reference sample to create an artificial organ was used by Bartrum et al. (1974) who called it the *reference organ method* This method has been used mostly in rather big laboratory animals such as sheep dogs, lambs, rabbits etc. (Buckberg et al. 1971 Domenech et al. 1969 Makowski et al. 1968, Archie et al. 1973 Bartrum et al. 1974) Rats have been studied by using the microsphere method without reference organs (Mendell & Hollenber 1971 Sasaki & Wagner 1971 Swedman 1977 Lindell 1977) Malik et al (1976) introduced the reference organ method in small animals—rats

The aim of this study was to evaluate the reference organ method for determining cardiac output and organ flow using $^{99}Tc^{m}$ labelled microspheres in rats.

THEORETICAL CONSIDERATIONS

The rate at which radioactive indicator q_i (µCi) (in our case $^{99}Tc^{m}$ microspheres) accumulates in an organ is expressed by the following equation:

$$dq_i/dt = f \quad C_i(\ ) - g_i \quad D_i(\ ) = f \quad [C_i(t) \quad D_i(t)] \tag{1}$$

where f is the blood flow (ml·min) to the organ i and $C_i(t)$ the activity concentration of the radioactive indicator (µCi/ml) in the blood at time after administration, g $= f$ is the blood flow out of the organ and $D_i(t)$ the activity

1 Specific blood flow in different organs in $\min^{-1}$ per gram of tissue and cardiac output [illegible] per gram of tissue ($n=16$)

	ml $\min^{-1}$ g		Cardiac output distribution % g^{-1}	
	Mean	±S.E.	Mean	S.E.
[illegible] kidney	3.8	0.7	5.8	0.7
[illegible] dney	3.9	0.9	5.8	0.6
coronary	10.9	[illegible]	17.4	3.4
[illegible]	0.3	0.05	0.4	0.1
[illegible]	0.3	0.05	0.4	0.1
	0.8	0.1	1.3	0.3
[illegible]	1.2	0.2	1.9	[illegible]
	0.4	0.1	0.6	0.1
[illegible]al muscle	0.1	0.02	0.2	0.02
[illegible]	0.1	0.02	0.2	0.06
	0.03	0.01	0.05	0.01

[illegible]-1000000 in volume of 0.2 ml. The activity was [illegible] μCi/injection. The catheter and the stopcock were [illegible] flushed with 0.5 ml of physiological saline. [illegible] before injection the withdrawal pump was started and [illegible] reference sample was drawn during 60 [illegible]. During the procedure the heart rate and intraventricular or [illegible]rial blood pressure [illegible] as recorded.

[illegible] rat was killed instantly by intracardiac injection of [illegible]ted potassium chloride 60 [illegible] after injection of the [illegible]spheres. At autopsy all catheter positions were iden[illegible]. Whole organs or tissue specimens and reference samples (Table 1) were taken and weighed with a [illegible] P1210 scale, put into plastic counting tubes and [illegible] with water. To minimize geometrical errors the [illegible] height in the counting tubes was kept below 1.5 [illegible]. The samples were counted in [illegible] well-type scintillation [illegible]er ("Selectronic") with a 2 [illegible] 2 inch NaI^{Tl} crystal. [illegible] counter was pre-set to 1000 [illegible] or one million counts. [illegible] output was estimated in ml $\min^{-1}$ 100 g [illegible] weight and organ blood flow in ml $\min^{-1}$ g^{-1} ac[illegible] to equations 6 and 7.

[illegible]ULTS

[illegible] significant disturbances were recorded in ven[illegible] lar or arterial pressure or the heart rate during [illegible] experiments. Blood pressure before injection of [illegible] spheres was 116±8 mmHg. Heart rate was [illegible]. Corresponding values after injection [illegible] 117±9 mmHg and 350±11 respectively (mean [illegible] S.E.). The average cardiac output in the rat was [illegible]±2.5 ml min [illegible] per 100 g b.wt. The specific [illegible]d flow in different organs is summarized in [illegible] le 1. The scatter diagram of the specific blood [illegible] in the left and right kidneys is shown in Fig. 1. [illegible] correlation coefficient is very close to 1 which

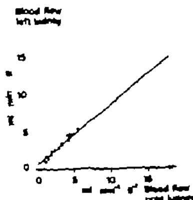


Fig. 1. Blood flow right/left kidney ml $\min^{-1}$ g. The equation of the regression line is $=0.8x+0.6$, the regression being significant $P<0.001$. The correlation coefficient is 0.992 and is highly significant $P<0.001$.

indicates that the microspheres are homogenously mixed with the blood. The specific blood flow through the heart muscle was 10.9 ml min [illegible] g [illegible]. There was no significant difference in specific arterial blood flow between the left, median, right or caudate liver lobes (Table 2). The mean specific blood flow was 0.7 ml min [illegible] g [illegible]. The pulmonary specific blood flow was 0.30 and equal in both lungs. No differences whatsoever were observed between the two strains of rats.

DISCUSSION

Determinations of cardiac output and organ blood flow utilizing the microsphere method and a reference organ have to fulfil certain criteria.

A uniform mixing of the microspheres with the blood before leaving the heart has been shown to be

Table 2. *Specific arterial blood flow in the liver in ml min [illegible] per gram of liver tissue and cardiac output distribution per gram of liver tissue ($n=16$)*

	ml min g^{-1}		Cardiac output distribution. % g	
	Mean	±S.E.	Mean	±S.E.
Left lobe	0.6	0.1	1.2	0.2
Median lobe	0.6	0.1	1.1	0.3
Right lobe	0.7	0.1	1.3	0.2
Caudate lobe	0.7	0.1	1.2	0.2
Total liver	0.7	0.1	1.2	0.2

concentration in the outflowing blood. The total amount of administered radioactive indicator Q can be expressed as the sum of radioactive indicator entering all organs and tissues in the body. Provided the radioactive indicator leaves the heart completely mixed with the blood the concentration of radioactive indicator entering all organs and tissues in the first circulation can be assumed to be the same, i.e. $C = C_i$.

In our case with the microspheres we integrate over an interval of several cycles of circulation. Thus the small fraction of untrapped microspheres in the first cycle is trapped in the subsequent cycles. However, the majority of the microspheres is trapped already at the first passage through the lungs. The amount of radioactive indicators in organ i after τ min is thus,

$$q = f \int^{\tau}[C(t) - D(t)]\,dt = f \int^{\tau}[C(t) + D(t) - D(t)]\,dt = f \int C(t)\,dt \qquad (2)$$

and in total body

$$Q = \Sigma_i q = \Sigma_i f \int^{\tau} C(t)\,dt = F \int^{\tau} C(t)\,dt \qquad (3)$$

where F is the sum of all individual flow rates which is equal to cardiac output (ml/min). Combining these two equations the following relation is found

$$Q/F = q_i/f = \int^{\tau}_{0} C(t)\,dt \quad [\mu\text{Ci} \cdot \text{min/ml}] \qquad (4)$$

If a reference blood sample r is collected from a peripheral artery at a known constant withdrawal rate f_r the cardiac output F can be calculated from the relation

$$\text{"Cardiac output"}\ F = f_r \cdot Q/q_r \qquad (5)$$

where f_r is known (0.5 ± 0.005) ml/min, Q is known from measurements of the total administered activity (μCi) and q_r is the activity (μCi) measured in the reference sample.

According to Buckberg et al. (1971) the number of microspheres in the samples are Poisson distributed, thus the standard deviation is $\sqrt{\bar{X}}$. $\bar{X}$ is the average number of microspheres in the sample. A 95% confidence interval can then be estimated as $\bar{X} \pm 1.96\sqrt{\bar{X}}$.

If we want to keep the variation of the number of microspheres in the sample within 3% at 95% confidence level we can write

$$\sqrt{\bar{X}} = 0.03\,\bar{X} \Rightarrow \bar{X} = 4000$$

Thus we want to have at least 4000 microspheres in each sample.

In order to minimize the influence of the animal weight on the results we calculated the specific blood flow φ_i for each organ and tissue i of weight m_i

$$\varphi_i = f_i/m_i = f_r/q_r \cdot q_i/m_i \qquad (6)$$

The cardiac output was normalized to ml/min per 100 g. M is the weight of the rat.

$$\Phi = (F/M)\,100 = f_r/q_r \cdot Q/M \cdot 100 \qquad (7)$$

In the reference organ method described the sample is drawn at a constant rate f_r. According to Buckberg et al. (1971) 1% of cardiac output can be safely drawn within 1 min without disturbing blood pressure and cardiac output.

In a rat with an assumed cardiac output of 50 ml/min the reference sample should thus be drawn at a rate of 0.5 ml/min. In order to obtain a minimum of 400 spheres in most samples an injected number of 400000 spheres is needed. In some small samples of 0.1 g of tissue with low blood flow, however, the number of spheres can be as low as 200–400 which in this case gives a precision of 10–15% in the number of spheres. The following formula can be used to calculate the number of spheres needed to obtain at least 400 in the tissue samples with the lowest activity

$$X = \frac{F \cdot 400}{f_i \cdot m_i} \text{ (Buckberg et al. 1971)}$$

F being the cardiac output ml/min and f_i the flow rate of the organ and m_i the sample weight.

MATERIALS AND METHODS

16 Wistar and Lister hooded rats of both sexes, weighing 200–250 g, were used for the experiment. Pentobarbital anesthesia was administered by intraperitoneal injection, 30 mg/kg in a 6 mg/ml solution. In supine position a Portex polyethylen catheter with an outer diameter of [illegible] mm was introduced into the right carotid artery and progressed down into the left ventricle. The catheter was connected to a transducer (Elema-Schonander EMT [illegible]) to record blood pressure and heart rate. The position of the cardiac catheter was corrected according to the pressure curve. Another catheter of the same size was introduced into the left femoral artery and progressed to the abdominal aorta or the common iliac artery. It was connected to a withdrawal pump (Harvard type) which was set at a flow rate of $f = 0.5$ ml/min with an accuracy of 0.1%. This pump suctioned the reference sample.

Dextran microspheres (Tracer Sephadex [illegible], Pharmacia Fine Chemicals, Uppsala, Sweden) were used. These microspheres consist of crosslinked Dextran and can be labelled with any radionuclides. The size of the microspheres was 15 ± 3 μm. The relative density was 1.1 ± 0.02 g/ml (closely approximating with that of blood cells). Sedimentation rate was [illegible]. The labelling of the microspheres was performed with ^{99}Tc^m pertechnetate using stannous-chloride as a reducing agent. After the microspheres were swollen at least one hour in trisbuffer a mixture of 0.5 ml ^{99}Tc^m pertechnetate and [illegible] ml 4 mM $SnCl_2$ (in 1 M NaCl at pH 1) was added. The ampule was shaken for 5–10 min. After washing and suspension in 10% Ficoll 70 (Pharmacia Fine Chemicals, Uppsala, Sweden) solution the ^{99}Tc^m labelled microspheres were ready to use. The labelling yield was more than 99%. No ^{99}Tc^m activity was released from the microsphere during 4 hours leaching in 10% Ficoll. After vigorous shaking of the syringe the labelled spheres were injected into the left ventricle within 10 s. The amount of microspheres injected [illegible] each rat was [illegible]

des of ^{99}Tc^{m}—short half life (6 h)—no corpus-
: radiation and high yield of easily detectable
na radiation (140 keV) give low radiation, ab-
ed dose to tissue and good in vivo detecta-
y by external measurements, i.e. using a scin-
on camera. It may therefore be possible to use
labelled degradable microspheres and the
rence organ method for in vivo blood flow deter
ation in humans. This will be further evaluated
rder to find if this is possible in clinical prac

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of great importance by Buckberg et al (1971) Domenech et al (1969) Neutze et al (1968) in larger animals and by Sasaki & Wagner (1971) in rats That a recirculation of spheres does not take place to any significant degree and that spheres are extracted completely during the first transit of the organ has been shown for spheres of various sizes (50 μm–15 μm) by Wagner et al (1969) Kaihara et al (1968) Mendel & Hollenberg (1971) Bartrum et al (1974) and Svedman (1977) However some shunting takes place in the ears and feet of the rabbit (Wagner et al 1974) and some in the kidneys of the rat (Svedman 1977) In the rat 2% of Tracer Sephadex® 15 μm spheres may be shunted to the lungs (Svedman 1977)

If the catheter is placed in the ventricle coronary blood flow studies seem to be less fit for the microsphere method Atrial catheters give a more uniform mixing at the orifices of the coronaries (Kaihara et al 1968) Thus the coronary flow in our series may be of less significance In the present series the mixing of the radioactive microspheres seemed to be adequate according to Fig. 1 where the renal flow through the two kidneys showed a correlation coefficient very close to one These measurements can be used as a test of homogenous mixing with the blood (Warren & Ledingham 1974) The activity in the lungs which represents the flow of the bronchial arteries and shunted spheres 0 3 ml min^{-1} g corresponds to less than 1% of cardiac output. This indicates a low degree of shunting.

In our series we used crosslinked dextran microspheres (Tracer Sephadex® 15 μm) labelled with $^{99}Tc^{m}$ The labelling was very efficient and stable which is of great importance in this type of study The biological activity and the physiological effects and characteristics of the microspheres have been thoroughly studied (Svedman 1977) The average size 15 μm is probably optimal for blood flow studies in small animals as a large number of spheres can be injected without causing disturbances in circulation or homeostasis The streaming characteristics of the microspheres are also uniform according to Phibbs & Dong (1970) In the present investigation no side effects were observed with Tracer Sephadex® spheres as measured by heart rate or blood pressure in spite of the great number of spheres injected (800 000–1 000 000)

Sasaki & Wagner (1971) found no effect on the distribution of blood flow in the brain after occlusion of one carotid artery in the rat With liga both carotid arteries there was a significa crease of cerebral blood flow and increase of t pressure but the blood flow to the rest of the was unaffected (Malik et al 1976) In our seri changes were registered in general behaviour pressure or heart rate after ligation of one ca artery The effect on blood flow was not s

The number of spheres was calculated w reasonable precision for determining low flo muscle skin and cartilage The determin cartilage flow must however be regarded somewhat less precise because of low w weight and low flow

The specific flow values in anesthetized ra ported in the series correspond well to resu others when they are normalized to percent of ca diac output which is the most common way to sent this kind of data (Saperstein 1958, 1960 M dell & Hollenberg 1971 Sasaki & Wagner 19 Malik et al 1976 and Svedman 1977). The relia of the microsphere method compared to ^{86}R been studied by Mendell & Hollenberg (197 Svedman (1977) Their conclusion was that nary circulation is best studied by soluble ind and preportal organs liver lung and brain best by microspheres

Cardiac output has been studied in bigger a by the reference organ method and the relia the method is well documented (Makowski 1968 Buckberg et al 1971 Mendell & Holl 1971 Archie et al 1973 Bartrum et al 197 Segedahl & Svanes 1977) The presented val cardiac output correspond well to determinatio rats made with other methods indicator d techniques using ^{86}Rb ^{42}K or direct Fick m (Sapirstein 1958 1960 Bullard 1956 and Bl al 1950) Malik et al (1976) using a s technique as ours using 15 μm microsphe belled with ^{85}Sr measured a cardiac output of 27 8±2 3 ml min 100 g b wt

The reference organ technique using radio microspheres as a tracer is a theoretically s method It may raise practical experimental lems that have to be overcome in order to o accurate results with good reproducibility present work shows that the reference organ te using $^{99}Tc^{m}$ labelled Tracer Sephadex® mi spheres can be used in the rat with good prec for determining cardiac output organ and t blood flow The favo[illegible] do

...ies of $^{99}Tc^m$—short half life (6 h)—no corpus... radiation and high yield of easily detectable ... radiation (140 keV) give low radiation, ab...ed dose to tissue and good in vivo detecta... by external measurements, i.e. using a scin...on camera. It may therefore be possible to use ... labelled degradable microspheres and the ...ence organ method for in vivo blood flow deter...tion in humans. This will be further evaluated ...der to find if this is possible in clinical prac...

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he time course of the latency relaxation as a function f the sarcomere length in frog and mammalian muscle

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BARTELS, E. M., SKYDSGAARD, J. M. & STEN-KNUDSEN, O.: The time course of the latency relaxation as function of the sarcomere length in frog and mammalian muscle. Acta Physiol Scand 1979 106, 129–137. Received 10 Oct. 1978. ISSN 0001-6772. Department of Biophysics, Panum Institute, University of Copenhagen, Denmark.

In comparative study the isometric twitch tension and the latency relaxation were correlated to the sarcomere length in frog and mammalian muscle, the latter only in the length range from 2.4 to 3.1 μm since at higher degrees of stretch the sarcomere lengths became increasingly non-uniform along the fibres. The location of the triads in mammalian muscle fibres was examined by means of electron microscopy. During stretch the location of the triads was gradually changed from the overlap zone at sarcomere lengths below 2.6 to 2.7 μm to the I-band at sarcomere lengths above 3.0 to 3.1 μm, their centres (T-tubules) being equally distributed between the overlap zone and the I-band at sarcomere lengths around 2.9 μm. In both types of muscle the maximum amplitude of the latency relaxation (of about equal relative size) occurred at a sarcomere length of about 3.1 μm, and both twitch tension and latency relaxation were dependent upon the presence of a zone of overlap between the thin and thick filaments. In neither of the two types of muscles did the time, t, from stimulation to the onset of tension drop depend upon the sarcomere length. At room temperature (22°C) t was about 1 ms in mammalian muscle and ms in frog muscle. In mammalian muscle the time, t_p, from the stimulus to the maximum drop in tension and the time, t_n, to positive tension development were both substantially uninfluenced by changes in sarcomere length in the range 2.4 to 2.9 μm, whereas in frog muscle both t and t increased linearly with increasing sarcomere length in the above range. These findings are discussed in the light of the different locations of the triads in frog and mammalian muscle. It is concluded that the theory of Sandow (1966)—extended by Mulieri (1977)—and that of Haugen & Sten-Knudsen (1976) which both have the virtue of being able to account for the increase of the latent period with stretch in frog muscle, also would be applicable to mammalian muscle provided that a time lag of 0.5 to 1.0 ms exists from the time of the binding of Ca^{++}-ions to the troponin molecules inside the zone of overlap until the attached cross bridges start to move and develop tension.

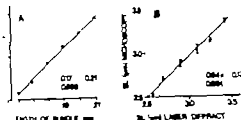
Key words: Latency relaxation, mammalian muscle, triads

atency relaxation (Sandow 1944) is the small tension drop which under isometric conditions precedes the development of contraction force proper. The underlying cause of latency relaxation is still unknown but it is generally considered as a reflection of some event in the excitation–contraction process. Recently Haugen & Sten-Knudsen (1976) observed that concomitant with the tension drop there is a small elongation of the sarcomeres. They ward the hypothesis that this elongation was a minute lengthening of the thin filaments of the conformational change upon the of the cal um ions to the troponin molecules (Haselgrove 1973). According to this hypothesis the falling phase of the latency relaxation should reflect the diffusion process of the Ca^{++}-ions from their time of liberation upon activation of the T-tubules until they reach the zone of overlap between the thin and thick filaments. In frog skeletal muscles T-tubules are located adjacent to the Z lines and, accordingly, the distance the Ca^{++} ions liberated have to travel by diffusion to reach the zone of overlap increases when the sarcomere length is increased by stretching the muscle. The hypothesis of Haugen & Sten-Knudsen (1976) therefore accounts for the well established

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1 Example of fibre bundle from rat gracilis muscle sarcomere length is linearly related to the fibre ... over the range of sarcomere lengths of 2.5 to 3.5 (A). The sarcomere length (SL) measured from laser ... action as function of the length of the fibre bundle. ...orrelation between values of sarcomere length obtained from laser diffraction and values of sarcomere length ...sured by light microscopy (verage ± S.D 3–5 meas... ...ents along the bundle). The equations obtained by regression are given in each diagram. correlation ...ficient.

...mation of the sarcomere length

...estimation of the sarcomere length in frog toe muscle ...means of laser diffraction technique has been described ...(...ic et al. 1976). The method could be used also in ...mmalian muscle Fig 1 shows comparisons between ...es obtained by the laser diffraction technique and ...es obtained by means of light microscopy of fibre ...the portions (cf fibre bundle preparation 2). A linear ...tionship between fibre length and sarcomere lengths ...seen in the range between 4 and 3 6 µm. The light ...oscopy as performed as follows Using a Zeiss ...ified WL microscope provided with water immer... ...on lens (Zeiss, 40 N A 0.75) microphotographs were ...en of 3–5 spots along the living fibre bundles at each ...stened length The sarcomere length was determined ...measuring in Recordak aperture card reader the ...gth of 20–30 sarcomeres in series and calculating the ...erage length using for comparison photograph of ...ibrated scale (100 divisions per mm)

...tron microscopy of rat muscle

...re bundles from M. semitendinosus of the rat were ...sected in oxygenated Ringer's solution. The bundles ...re stretched to arious lengths, and each bundle as ...ntained at a fixed length by Teflon holder during ...ation in glutaraldehyde (2 5% 120 min) and osmium ...roxide (2% 90 min, 20°C) both buffered in 0.1 M ...ium cacodylate at pH 7 4. After dehydration in ...aded series of ethanol followed by propylene xide and ...ropylene oxide plus Epon, the preparation was embed... ...ed in Epon and cut by an LKB Ultrotome I in 50–190 nm ...tions. The specimens were poststained by 4% unc... ...anyl acetate according to Weinstein et al. (1963) at 60°C ... 30 min and 0.4% lead citrate at room temperature ... 3 min. For electron microscopy an AEI-Corinth 275 ...croscope was used.

RESULTS

1 The distribution of sarcomere lengths in frog and mammalian muscle at arious degree of stretch

It was essential for this investigation that the sarcomere lengths were fairly uniformly distributed along the fibres at each muscle length. To find the range of muscle lengths where this requirement was fulfilled the preparation was scanned along its length with the laser beam at each degree of stretch The measurements of the sarcomere lengths were taken during consecutive stretches and relaxations of the muscles.

In *the f og toe muscle* the sarcomere length was proportional to the muscle length at sarcomere lengths between ...3 and 3 4 µm. In this range the differences between the highest and the lowest sarcomere length measured along the muscles were less than 0.1 µm, the fibre ends being excluded (Huxley & Peachey 1961) The muscles showed functional characteristics similar to those found in single fibres by Haugen & Sten-Knudsen (1976) The isometric twitch and the tension relaxation passed through their maxima at sarcomere lengths slightly below ...4 µm and at 3 10±0.15 µm (S.D. 43 obs. in 12 muscles) respectively both extrapolating to zero at a sarcomere length about 3 65–3 70 µm.

The *mammalian muscle* preparations were usually slack at sarcomere lengths below ...4 µm. When the muscles were stretched the sarcomere lengths remained fairly uniformly distributed along the lengths of the muscles up to an average sarcomere length of 3 1 µm (cf Fig.) When stretched further irregularities in the distribution of sarcomere lengths appeared In fibre bundles the laser beam revealed alternating parts with sharp and disappearing diffraction spectra, when the sarcomere length was above 3 1 µm and the bundles developed isometric tension which extrapolated to zero at sarcomere lengths varied from 3 7 to above 4 0 µm. A small M gracilis was superior to the fibre bundles in reproducing the length–tension curves provided the muscles were not stretched to sarcomere lengths above 3 2 µm. Furthermore M gracilis offered a reasonable explanation to the puzzling finding of tension development in fibre bundles in which several cross-sections had sarcomere lengths far above 3 7 µm. Coincident with an almost abrupt increase of the resting tension, M. gracilis showed an in-

observation that in frog skeletal muscle the time from the stimulation to the first drop in tension is independent of the sarcomere length whereas the time to the maximum drop in tension is a linear function of the sarcomere length *s* at least in the range 2 2 μm<s<3 1 μm (Guld & Sten-Knudsen 1960 Mulieri 1972 Haugen & Sten-Knudsen 1976) In mammalian muscles the T tubules are known to be located near the zone of overlap (Porter & Palade 1957) The distances which the Ca^{2+} ions have to travel in these muscles to reach the zone of overlap are shorter and do not depend upon the sarcomere length in the same way as in the frog muscles According to the hypothesis of Haugen & Sten-Knudsen (1976) one should therefore expect that the time course of the latency relaxation depended differently upon the sarcomere length in mammalian and in frog muscles Therefore to put the above mentioned hypothesis to a test we have examined the dependence of the latency relaxation upon the sarcomere length in mammalian muscles As we were not able to prepare single fibres from mammalian muscles the investigation was carried out as a comparison between whole muscles of almost equal size from frog and mice or on rat fibre bundles A preliminary report of this investigation was given at a meeting in Bressanone 1976 held by the European Muscle Club

METHODS

Preparations

The experiments were performed on fibre bundles or whole muscles with about uniform fibre lengths throughout the specimen at each degree of stretch While this requirement could be reasonably fulfilled in frog muscle at sarcomere lengths between 2.3 and 3 4 μm non-uniformity of sarcomere lengths developed in mammalian muscle preparations at sarcomere lengths above 3 1–3 2 μm.

Both fibre bundles and whole muscles were prepared Fibre bundles can be fixed rapidly for electron microscopy and were used for the examination of the location of the triads at various sarcomere lengths in mammalian muscle The bundles were also used for the investigation of the latency relaxation but the length range in which the sarcomeres had uniform lengths was not wider in fibre bundles than in whole muscles. Therefore small undamaged whole muscles were considered more reliable for the study of latency relaxation as a function of sarcomere length.

Whole muscle

1 From the frog (*Rana temporaria*) the M extensor long. dig. IV (toe muscle) was isolated and both tendons were fitted with light steel hooks for attachment to the transducers The dissection and the experiments were carried out in a Ringer s solution containing (mM): NaCl [illegible] ? $CaCl_2$ 1 8, Na_2HPO_4 0 9 KH_2PO_4 [illegible] *d* Tubocurarine 1 μg/cm^3 was added to the solution

2. *The M gracilis anticus* from the mouse [illegible] neighbouring tissues and the portions of tibia and pelvis to which the muscle inserts were removed under [illegible] and transferred to a special chamber for final [illegible] The fascia was removed as completely as possible without damaging any fibres. For transducer attachment a loop of platinum wire (diameter 0 ? mm) was fixed to the [illegible] of tibia, and a silk loop was fixed to the fragment of pelvic bone The final preparation appeared as a [illegible] rectangular strip about 0.5 mm thick 1 5–2 mm [illegible] and 15–18 mm long at the resting length

M gracilis and the fibre bundles described in the following were dissected and investigated in a Ringer s solution containing (mM) NaCl 140 KCl 5 $CaCl_2$ [illegible] $MgCl_2$ 1 Na_2HPO_4 1 1 NaH_2PO_4 0.4 (pH 7 4) glucose [illegible] Ringer s solution was oxygenated during the [illegible] and *d* Tubocurarine 1 μg/cm^3 was present during the experiments.

Fibre bundle preparations

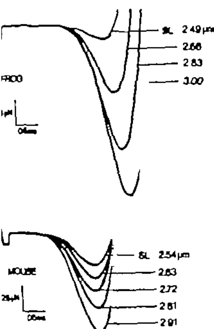
1 Narrow fibre bundles from *M semitendinosus* [illegible] *adductor long* in the rat were prepared at full length fitted with silk loops tied to the tendinous ends [illegible] superficial fibres were always damaged in this type preparation

These bundles functioned for about two hours [illegible] the maximum isometric twitch began to decline. [illegible] were used for both electron microscopy (see below) [illegible] for registration of the latency relaxation at various sarcomere lengths.

2 *Portions—about 1.5 cm long—of fibre bundles* [illegible] gracilis muscle could be prepared and isolated after [illegible] tion without any damage to the isolated fibre portion taking advantage of the divisions occurring naturally [illegible] muscles The ligature was placed outside but very close [illegible] the dissected portion the ends of the portion thus [illegible] protected by neighbouring fibres and connective tissue These bundle portions had uniform sarcomere lengths [illegible] the range from 2.5 to 3.5 μm, and they were used for examination of the degree to which sarcomere length estimated by the laser diffraction technique agreed [illegible] sarcomere lengths estimated by light microscopy (see below)

Experimental procedure

All experiments were carried out at 20–22°C [illegible] technique described earlier in detail (Bartels et al. [illegible] The preparation was mounted between two strain gauge transducers for simultaneous recording of the [illegible] tension (resting tension and isometric force) and the latency relaxation The muscle length was altered by [illegible] the arm of the manipulator holding the tension transducer At each muscle length the sarcomere length was measured and the preparation was then given an [illegible] stimulation (transversely) with a diphasic current [illegible] usually of 0.5 ms duration



3 The time course of the latency relaxation at var sarcomere lengths in whole muscles from the frog (M. long dig IV) and the mouse (M gracilis antic). ecdings were superimposed at various sarcomere hs (SL in µm are indicated at each trace. Note the rent tension scales in the two sets of recordings).

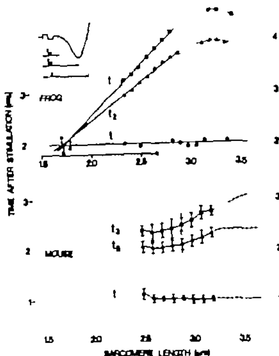


Fig 4 The influence of the sarcomere length on the time of onset of the latency relaxation, *t* (filled circles) the time to maximum tension drop, *t* (triangles) and the time to development of positive tension *t* (filled squares) in whole muscles from frog and mouse.

Upper part: results from one frog toe muscle =1.50±0.37 µm: intersection between the mean level for *t* and the regression line for *t* *b*=1.55±0.33 µm: intersection between the mean level for t and the regression line for *t* =1.61±0.37 µm: intersection between the regression lines for *t* and *t* (A erage±S.D 43 obs on 13 muscles)

Lower part: *t* t_2, and *t* (average ± S.D) from 9 M gracilis antic. from mice

limited to sarcomere lengths between 2.4 and µm. This limited range of sarcomere lengths had the advantage of being the range in which resting tension was small and accordingly nges in the parallel elasticity has a relatively all influence on the time course of the latency xation.

The time course of the lat ncy elaxation function of the sarcomer le gth in frog d mammalian hole muscl

amples of the superimposed recordings of the ency relaxation at arious sarcomere lengths in og toe muscle and in M gracilis in the mouse are own in Fig 3 All the times were measured from start of the stimulating pulse. In mammalian uscle the time to the onset of the tension drop was out half of that in frog muscle However the ain difference between the two sets of recordings as that in mammalian muscle as contrasted to frog uscle, the duration of the latency relaxation was most uninfluenced by changes in sarcomere length This is further illustrated in F g. 4 In agreement with earlier findings (Guld & Sten-Knudsen 1960) the pattern in frog toe muscle was found similar to that in single fibres (Mulieri 1972, Haugen & Sten-Knudsen 1976) While the time, *t* from stimulation to onset of the tension drop seems uninfluenced by the sarcomere length both the time t_2, for maximum tension drop and the time t_3, for the development of positive tension increased linearly with increasing sarcomere length in the range 2.4–3.2 µm (Fig 4 upper part) The regression lines for *t* and *t* intersected with the mean level for *t* at a sarcomere length around 1.5–1.6 µm being close to the length of the thick filament. At sarcomere

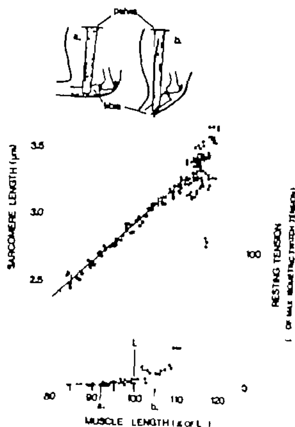


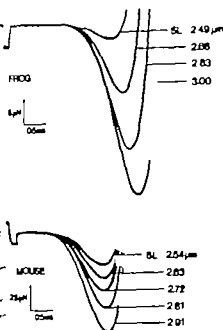
Fig 2 To illustrate the increasing non-uniformity of the sarcomere lengths with increasing stretch of 9 gracilis muscles from mice Abscissa, muscle length as a percentage of length L_o at which maximum isometric twitch tension developed L corresponds to a sarcomere length of about 2.9 µm. Right ordinate resting tension as a percentage of maximum twitch tension Left ordinate sarcomere length in µm Open circles indicate that the sarcomere lengths were uniformly distributed Each point represents the average from three measurements along each muscle Filled circles all measurements from the proximal the middle and the distal third of each muscle On the abscissa (*a*) and (*b*) indicate the approximate limits of muscle length in the intact animals. The appearance of M gracilis in situ at these limits of length are shown in the insert sketch

creasing non-uniformity of the sarcomere length when stretched to sarcomere lengths above 3 ? µm (Fig ?) Simultaneously the isometric twitch which was maximal at a sarcomere length of about 2 9 µm decreased linearly with increasing muscle length and a twitch could still develop when the proximal third of the muscle showed sarcomere lengths up to 4 µm However when correlated to the sarcomere length of just the distal third of the muscle the isometric twitch extrapolated to zero at sarcomere lengths around 3 5–3 6 µm which suggests that the parallel elasticity (connective tissue etc) of the extremely stretched fiber fractions acted as a series elastic element between the distal contracting fractions and the proximal tendon. After a stretch beyond 3 ? µm of the shortest sarcomere the length–tension curves changed irreversibly, calling a similar finding of Bahler et al. (1968) isolated rat gracilis muscle stretched beyond [illegible] of the resting length Apparently the connective tissue is woven around the fibres in a way that brings about an inhomogenous strain, the various parts of the fibres not being stretched to the same degree

The amplitude of the latency relaxation was maximal at a sarcomere length of about 3 1–3 [illegible] in mammalian whole muscle The maximum amplitude was of about the same relative size in both frog and mammalian muscle The maximum amplitude expressed per thousand of the maximum isometric twitch tension was on average 1 1 in [illegible] muscle 2.5 in mouse muscle and 1 7 in rat fibre bundles At further stretch of mammalian muscle the lengths with non-uniform distribution of the sarcomere length no consistent pattern of the amplitude as a function of muscle length could be obtained However when a linear decline was observed occasionally both the isometric twitch tension and the amplitude of the latency relaxation extrapolated to zero at about the same muscle length and a latency relaxation was never observed unless accompanied by a twitch

If the sarcomere lengths were restricted to the range [illegible] 4–3 1 µm (Fig. 2) the sarcomere length was proportional to the muscle length The difference between the highest and the lowest sarcomere length observed along the muscles at each length was on the average 0 07 µm (S D = ±0.05 µm, [illegible] obs in 9 muscles) By transverse scanning with the laser beam it was found that the sarcomere length in M gracilis at the resting length varied in a regular way being about 5% higher in the anterior edge than in the posterior edge of the muscle in its rectangular form A possible explanation is that the muscle—being among others a flexor of the knee—has optimal fibre lengths when it is non-rectangular at stretched knee (Fig ? inserted illustration) However this non-uniformity played a minor role in this investigation because the time course of the latency relaxation turned out to be almost independent of the sarcomere length in the range 2.4–3.1 µm

In accordance with these findings the following comparison between frog and mammalian muscle



...3 The time course of the latency relaxation at var... sarcomere lengths in whole muscles from the frog (M. ...long. dig. IV) and the mouse (M. gracilis antic.). ...rdings were superimposed at various sarcomere ... the (SL in µm are indicated at each trace. Note the ...ent tension scales in the two sets of recordings)

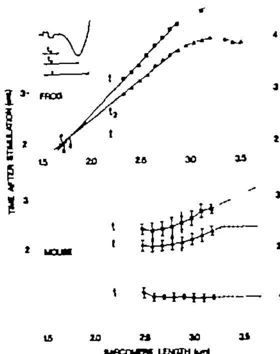


Fig. 4. The influence of the sarcomere length on the time of onset of the latency relaxation, t (filled circles) the time to maximum tension drop, t (triangles) and the time to development of positive tension t (filled squares), in ...hole muscles from frog and mouse.

Upper part: results from one frog toe muscle. ...=1.50±0.37 µm: intersection between the mean level for t and the regression line for t_a. b=1.55±0.33 µm. intersection between the mean level for t and the regression line for t_a. =1.61±0.37 µm: intersection between the regression lines for t and t_a. (A erage±S.D. 43 obs. on 13 muscles.)

Lower part: t, t_a and t (a erage ± S.D.) from 9 M. gracilis antic. from mice

...mited to sarcomere lengths between 2.4 and ...m. This limited range of sarcomere lengths ...ad the advantage of being the range in which ...esting tension was small and accordingly ...ges in the parallel elasticity has a relatively ...l influence on the time course of the latency ...ation

...he time course of th latency relaxation ... function of the sarcomere length in frog ... mammalian hole mus l

...mples of the superimposed recordings of the ...ncy relaxation at various sarcomere lengths in ... toe muscle and in M. gracilis in the mouse are ... in Fig. 3. All the times were measured from ... start of the stimulating pulse. In mammalian ...scle the time to the onset of the tension drop was ...out half of that in frog muscle. However the ...ain difference between the two sets of recordings ...as that in mammalian muscle as contrasted to frog ...uscle the duration of the latency relaxation was ...most uninfluenced by changes in sarcomere length. This is further illustrated in Fig. 4. In agreement with earlier findings (Guld & Sten-Knudsen 1960) the pattern in frog toe muscle was found similar to that in single fibres (Mulieri 1972, Haugen & Sten-Knudsen 1976). While the time, t, from stimulation to onset of the tension drop seems uninfluenced by the sarcomere length, both the time, t_a, for maximum tension drop and the time t_a, for the development of positive tension increased linearly with increasing sarcomere length in the range 2.4–3.2 µm (Fig. 4, upper part). The regression lines for t and t intersected with the mean level for t at sarcomere length around 1.5–1.6 µm, being close to the length of the thick filament. At sarcomere

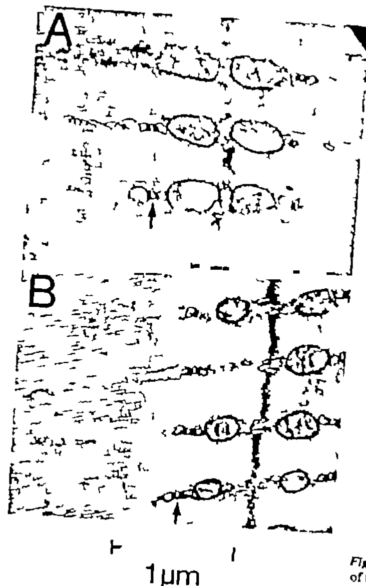


Fig 5 Electronmicrographs of the rat semitendinosus muscle Magnification ×30 000. T-tubules shown by arrows (A) Sarcomere length ~3 µm T-tubules located in the A-band (B) Sarcomere length 3.3 µm T tubules in the I-band

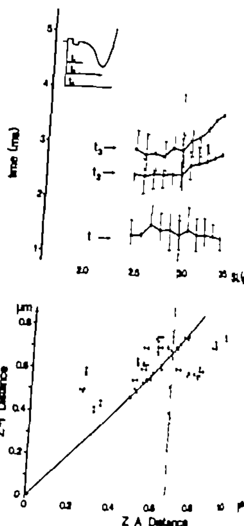


Fig 6 Duration of the latency relaxation and the location of the T-tubules at various sarcomere lengths in fibre bundles from rat skeletal muscle

Upper part. The times to onset (t) to maximum and to termination (t_3) of the latency relaxation as a function of sarcomere length (SL) Ordinate time. Abscissa, common to upper and lower part sarcomere length (µm) Average±S.D 37 obs. in 19 fibre bundles

Lower part Location of the T-tubules at various sarcomere lengths 189 measurements. Ordinate, distance (Z–T) between the Z line and the T-tubules (µm). Lower abscissa, distance (Z–A) between the Z-line and myosin filaments (µm) For further explanation see text

lengths above 3.2 µm both t_2 and t_3 leveled off and could eventually decrease In mammalian muscle (Fig. 4 lower part Fig 6 upper part) the time course of the latency relaxation as a function of sarcomere length differed markedly from the pattern observed in frog muscle in the range of sarcomere lengths from 2.5 to 2 9 µm. Both t_1 and t_2 were found substantially independent of the sarcomere length and t_3 showed only a slight increase as the sarcomere length was increased from 2 5 to 2.9 µm At sarcomere lengths from 2 9 µm to about 3 µm both t_2 and t_3 tended to increase in mammalian muscle

3 *The location of the triads at various sarcomere lengths in mammalian muscle*

The location of the triads adjacent to the overlap zone between thick and thin filaments in mammalian muscle is well known (Porter & Palade 1957) To our knowledge there was no information on the displacement of the triads relative to the overlap zone as the sarcomere length changes Since the possibility of such displacement is important we carried out a systematic examination of the

on of the triads in mammalian muscle fibres at mere lengths from ..0 to 3 7 μm The results a in Figs. 5 and 6 were obtained with rat fibre les that were originally used for both the anical and the morphological studies of mam- n muscle. The micrographs in Fig. 5 show aples of sarcomeres at two different lengths. At comere length of 2.3 μm the triads were found e overlap zone between the thick and thin fila- ts, but at a sarcomere length of 3.3 μm the s were located in the I-band This behaviour is er illustrated in Fig. 6 (lower part) showing the lts of 189 measurements of the location of the bules at various degrees of stretch. At each omere length the distance between the Z-line the T-tubule (Z T Distance) was plotted against distance between the Z-line and the A-band A Distance). The solid line indicates those T les which at each sarcomere length are located he border of the overlap zone and the I-band. refore this line separates the observed T les into two groups. ( ) those situated in the rlap zone between the thick and thin filaments pos above the line) and (b) those situated out the overlap zone (region below the line) At -A)-distances below 0.5 μm corresponding to a mere length of 2.6 μm practically all T-tubules e found in the overlap zone At (Z–A)-distances ween 0 6 and 0 65 μm corresponding to sarco- e lengths between 2.8 and 2.9 μm about 50% of T-tubules were situated in the overlap zone At -A)-distances above 0 75 μm (sarcomere length: μm) corresponding to muscle lengths above the ximal length in the intact organism all T tubules med to have been moved out of the overlap ne. In other words, the T-tubules were found dominantly in the overlap zone at sarcomere gths below 2.8 μm, and predominantly in the nd at sarcomere lengths above ..9 μm

Fig. 6 (upper part) illustrates that in rat fibre ndles—like in mouse gracilis muscle—t seems to independent of the sarcomere length in the range 4–.9 μm, and that t tends to increase when the ndles were stretched to sarcomere lengths above 9 μm. This means that t tends to increase com- ing with displacement of the triads away from e overlap zone It should be kept in mind that ither in the fibre bundles nor in the whole muscle, uld the time course of the latency relaxation be orrelated to a well defined sarcomere length above rcomere lengths of 3 1 3.2 μm.

DISCUSSION

Latency relaxation was first observed in mam- malian muscles by Goepfert & Schaefer (194.) Their findings were later confirmed by Abbott & Ritchie (1951) None of these investigators made a systematic study of the length dependence of the latency relaxation or did correlate it to sarcomere length. The attempt made in the present investiga- tion to extend the previous works by correlating the time course of the latency relaxation to sarcomere length was only partially successful since the dis- tribution of sarcomere lengths became increasingly inhomogeneous at higher degrees of stretch. For this reason results usable for the primary purpose of this investigation could only be obtained in the range of sarcomere lengths up to 3 1 3.2 μm. How- ever by relating the twitch tension to the shortest sarcomeres observed in the muscles under extreme degrees of stretch, contraction did not occur when overlapping between thin and thick filaments no longer existed Furthermore the latency relaxation usually came to a maximum at a sarcomere length of 3 1 μm or more and was not observed unless accompanied by a twitch. For these reasons we shall in the following assume that the same basic mechanism is responsible for the latency relaxation in both frog and mammalian muscles.

The main result of the present investigation was the finding of a different correlation in mammalian and in frog muscle between the sarcomere length and the time course of the latency relaxation: In mammalian muscle the time t from the stimulus to the maximum drop in tension and the time t_p to positive tension development were both substan- tially uninfluenced by changes in sarcomere length in the range 2.4–2.9 μm whereas in frog muscle both t and t increased linearly with increasing sarcomere length in the above range the last result being in agreement with previous observations (Guld & Sten-Knudsen 1960 Haugen & Sten- Knudsen 1976) It is natural to ask whether this difference could most easily be understood in terms of the different location of the triads in frog and mammalian muscle Among the various proposals put forward to account for the latency relaxation in frog muscle (Sandow 1947 A F Huxley 1957 Sandow 1966, H E. Huxley & Brown 1967 Hill 1968, Peachey 1968 Mulieri 1972, Haugen & Sten-Knudsen 1976) only the theory of Sandow (1966)—extended by Mulieri (1972)—and that of

Haugen & Sten-Knudsen (1976) attempt to account for the increase in the latent period with stretch in frog muscle Although differing as to the origin of the latency relaxation proper both theories explain the increasing latent period with stretch by the increasing distance which the Ca^{2+} ions liberated from the triads upon stimulation have to travel until they reach the overlap zone Accordingly both theories would have been invalidated if the time course of the latency relaxation had been found to depend upon the sarcomere length in exactly the same way in frog and mammalian muscle As it is the present results are not at variance either with the theory of Sandow (1966) or with that of Haugen & Sten Knudsen (1976) since in mammalian muscle the distance which the Ca^{2+} ions must travel to reach the overlapping filaments changes little with sarcomere length and consequently the time course of the latency relaxation should be almost invariant to changes in sarcomere length But this also applies to those theories which attribute the origin of the latency relaxation to processes located in the overlap region (Hill 1968 H E Huxley & Brown 1967 Wilkie personal communication) On the other hand these theories do not account for the increase in the latent period with stretch in frog muscle and it seems doubtful whether they will be capable of doing it in a straightforward manner (Haugen & Sten-Knudsen 1976) For this reason we may justifiably examine whether the location of the triads in mammalian muscle is compatible with the appearance of a latency relaxation according only to those theories simply accounting for the increase in latent period with stretch in frog muscle i e the theory of Sandow (1966) and that of Haugen & Sten Knudsen (1976)

When Ca^{2+} ions are liberated from the triads in mammalian muscle these ions enter almost simultaneously depending upon the exact location of the triads both the A I filamentary lattice in the overlap zone and the neighbouring thin filaments in the I band The last event is immaterial in the Sandow Mulieri theory where the latency relaxation is considered to be caused by a change in the compliance of the sarcoplasmatic reticulum resulting from the liberation of the Ca^{2+}-ions To obtain a latency relaxation of about the same size in both frog and mammalian muscle this theory would require a time delay of about 0.5–1 0 ms from the time the Ca^{2+}-ions enter the filamentary lattice in the overlap zone until the tension developing process starts, i e movements of the cross bri[illegible] (Fig. 3) Such a time lag is even more [illegible] needed to preserve the Haugen and Sten-Kn[illegible] theory According to this theory the origin of [illegible] latency relaxation is regarded to be an elong[illegible] of the thin filaments resulting from a conformat[illegible] change in the tropomyosin molecules upon [illegible] binding of the Ca^{2+} ions to the tropon[illegible] (Haugen & Sten-Knudsen 1976). The lengthen[illegible] of the sarcomere resulting from this elongation [illegible] considered mediated by an interfilamentary [illegible] coupling probably cross bridges which are attach[illegible] even in the resting state. Thus, according to [illegible] theory the Ca^{2+} ions entering the I-band are [illegible]sential in the development of the latency relaxa[illegible] To make this theory work also in mamma[illegible] muscle and to provide a latency relaxation of [illegible] the same size as in the frog it is again necess[illegible] that a sufficient time (about 0.5–1 0 ms) exist[illegible] the development of the conformational change [illegible] the tropomyosin molecules (Haselgrove 1973) at [illegible] I band before the cross bridges in the overlap [illegible] attach to the I filaments and start their tension de[illegible]veloping movements.

If only the results from mammalian muscle h[illegible] been available the Haugen & Sten-Knudsen the[illegible] would probably have been the last one to be [illegible]sidered if at all Granted that there is the abo[illegible] mentioned delay (at least 0.5 ms) the questi[illegible] remains which of the two theories is the most [illegible]plicable In this work there is no safe ground [illegible] prefer one to the other However compared [illegible] the Sandow Mulieri hypothesis that of Haugen [illegible] Sten Knudsen has the advantage of explaining [illegible] easily that latency relaxation does not devel[illegible] unless an overlap exists between the thick and th[illegible] filaments

The authors wish to acknowledge their indebtedness [illegible] Miss Elisabeth Kroger for devoting her skill to the prep[illegible]ration of the figures.

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Haugen & Sten-Knudsen (1976) attempt to account for the increase in the latent period with stretch in frog muscle Although differing as to the origin of the latency relaxation proper both theories explain the increasing latent period with stretch by the increasing distance which the Ca^{2+} ions liberated from the triads upon stimulation have to travel until they reach the overlap zone Accordingly both theories would have been invalidated if the time course of the latency relaxation had been found to depend upon the sarcomere length in exactly the same way in frog and mammalian muscle As it is the present results are not at variance either with the theory of Sandow (1966) or with that of Haugen & Sten-Knudsen (1976) since in mammalian muscle the distance which the Ca^{2+} ions must travel to reach the overlapping filaments changes little with sarcomere length and consequently the time course of the latency relaxation should be almost invariant to changes in sarcomere length But this also applies to those theories which attribute the origin of the latency relaxation to processes located in the over lap region (Hill 1968 H E Huxley & Brown 1967 Wilkie personal communication) On the other hand these theories do not account for the increase in the latent period with stretch in frog muscle and it seems doubtful whether they will be capable of doing it in a straightforward manner (Haugen & Sten-Knudsen 1976) For this reason we may justifiably examine whether the location of the triads in mammalian muscle is compatible with the appear ance of a latency relaxation according only to those theories simply accounting for the increase in latent period with stretch in frog muscle i.e the theory of Sandow (1966) and that of Haugen & Sten Knudsen (1976)

When Ca^{2+}-ions are liberated from the triads in mammalian muscle these ions enter almost simul taneously depending upon the exact location of the triads both the A I filamentary lattice in the over lap zone and the neighbouring thin filaments in the I band The last event is immaterial in the Sandow Mulieri theory where the latency relaxation is considered to be caused by a change in the com pliance of the sarcoplasmatic reticulum resulting from the liberation of the Ca^{2+}-ions To obtain a latency relaxation of about the same size in both frog and mammalian muscle this theory would re quire a time delay of about 0.5–1 0 ms from the time the Ca^{2+}-ions enter the filamentary lattice in the overlap zone until the tension developing process starts i e movements of the cross br[illegible] (Fig 3) Such a time lag is even more [illegible] needed to preserve the Haugen and Sten Kn[illegible] theory According to this theory the origin of latency relaxation is regarded to be an elong[illegible] of the thin filaments resulting from a conformat[illegible] change in the tropomyosin molecules upon binding of the Ca^{2+}-ions to the tropon[illegible] (Haugen & Sten-Knudsen 1976) The lengthe[illegible] of the sarcomere resulting from this elongation [illegible] considered mediated by an interfilamentary [illegible] coupling probably cross bridges which are att[illegible] even in the resting state Thus according to [illegible] theory the Ca^{2+} ions entering the I-band are [illegible] sential in the development of the latency relax[illegible] To make this theory work also in mamm[illegible] muscle and to provide a latency relaxation of [illegible] the same size as in the frog it is again [illegible] that a sufficient time (about 0.5–1 0 ms) exis[illegible] the development of the conformational chang[illegible] the tropomyosin molecules (Haselgrove 1973) [illegible] I band before the cross bridges in the overlap [illegible] attach to the I-filaments and start their tensio[illegible] veloping movements

If only the results from mammalian muscle [illegible] been available the Haugen & Sten-Knudsen the[illegible] would probably have been the last one to be [illegible] sidered if at all Granted that there is the ab[illegible] mentioned delay (at least 0.5 ms) the ques[illegible] remains which of the two theories is the mor[illegible] plicable In this work there is no safe ground [illegible] prefer one to the other However compared [illegible] the Sandow Mulieri hypothesis that of Haugen [illegible] Sten-Knudsen has the advantage of explaining [illegible] easily that latency relaxation does not dev[illegible] unless an overlap exists between the thick and [illegible] filaments

The authors wish to acknowledge their indebtedness [illegible] Miss Elisabeth Kroger for devoting her skill to the pr[illegible] ration of the figures.

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Physiol Scand 1979 106. 139–143

gional changes in monoamine synthesis in the developing ɩbrain during hypoxia

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HEDNER, T & LUNDBORG, P Regional changes in monoamine synthesis in the developing rat brain during hypoxia. Acta Physiol Scand 1979 106. 139–143 Received 11 Oct. 1978. ISSN 0001-6772. Department of Pharmacology University of Goteborg, Sweden.

4 14 and 28 days old rats were exposed to hypoxic environment of 6% O_2–94% N_2 for 30 min. Tyrosine hydroxylase and tryptophan hydroxylase activity was studied in different brain regions (hemispheres, striatum midbrain and brainstem) in vivo by measuring the accumulation of dihydroxyphenylalanine (Dopa) and 5-hydroxytryptophan (5-HTP) respectively after inhibition of aromatic L-amino acid decarboxylase with NSD 1015 Tyrosine and tryptophan levels in the different brain regions were measured simultaneously The tyrosine and tryptophan levels in the various brain parts were generally not influenced during exposure to hypoxia. Tyrosine hydroxylase activity decreased in most areas in the 4 and 14 days old rats, and all brain areas studied in the 28 days old rats. Tryptophan hydroxylase activity decreased markedly in all brain areas at all ages studied. It is concluded that the enzymes tyrosine hydroxylase as well as tryptophan hydroxylase seem to be equally affected during hypoxia in the different brain regions studied.

Key words Hypoxia, developing brain, monoamine synthesis

out all the various groups of monoamine—containing nerve cell bodies which are present in the adult rat brain are also present at a very early stage of development (Loizou 1969 1972) From then on, the levels of dopamine (DA) noradrenaline (NA) and 5-hydroxytryptamine (5-HT) increase with proceeding age and do not reach adult levels until several weeks after birth in the rat or as in the case of DA, not until adult life (Coyle 1973 Thornburg & Moore 1976) This sequence of events is probably a consequence of the centrifugal outgrowth of axons and terminals from the cell bodies (Loizou 1972)

The activities of the first and rate limiting enzymes of the catecholamine and indoleamine synthesis pathways, tyrosine hydroxylase and tryptophan hydroxylase respectively have been detected during late gestation (Coyle & Axelrod 1972 b) Analysis of the regional distribution of tyrosine hydroxylase and tryptophan hydroxylase in the rat brain indicate that the maturation of these enzymes proceed in a caudal to rostral direction (Coyle & Axelrod 1972 b, Deguchi & Barchas 1972)

Recent studies from these laboratories have demonstrated that in neonatal and developing animals hypoxia and/or anoxia are accompanied by marked acute alterations in the synthesis of the monoamine neurotransmitters DA NA and 5-HT (Hedner et al 1977 Hedner et al. 1977 b Hedner et al 1978). Additionally we have demonstrated that persistent biochemical and behavioral impairment occur after severe neonatal oxygen deprivation as measured as monoamine synthesis and conditioned avoidance response, respectively (Hedner et al. to be publ.)

As the maturation of the various monoamine neuronal pathways in the brain proceed in an asynchronous way the present study was undertaken in order to investigate possible differences in sensitivity to hypoxia between different brain regions

METHODS

Pregnant Sprague-Dawley rats (Anticimex Stockholm) were housed under regulated dark-light conditions (light period 6 a.m.–6 p.m.) in the department and the time of birth was noted within 12 h. At 4 14 and 28 days of age,

JLTS

ne and tryptophan levels

...ne and tryptophan levels tended to decrease ... arious brain areas with advancing age. Ex... e to hypoxia did not significantly alter the ...ne levels in the different brain parts except ... decrease noted in the brain stem at 14 days of ... Table 1). During 30 min of exposure to 6% ...1% N_2 no alteration in tryptophan levels was ... compared to controls (Table 2).

and 5-HTP accumulation after NSD 1015

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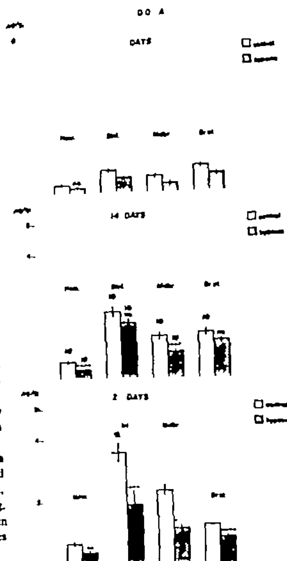


Fig. 1 Brain tyrosine hydroxylase activity in 4, 14 and 28 days old rats during hypoxia. Tyrosine hydroxylase activity was measured in various brain regions (*Hem* hemispheres, *Str.* striatum, *Midbr* midbrain, *Br.st* brainstem) by means of Dopa-accumulation after inhibition of aromatic L-amino acid decarboxylase with NSD 1015 injected subcutaneously 30 min before sacrifice. Shown are means ±S.E. Figures indicate the number of observations. Comparisons by *t*-test. $P<0.05$ $^{**}P<0.01$ $^{**}P<0.005$ $^{**}P<0.001$ — not significant

...SCUSSION

...perimental animal studies have shown that dif...ent forms of severe oxygen deprivation, such as ...poxia, anoxia or ischemia, have one common ...uropathological denominator (Brierly et al. 1973), ...ulting in a highly predictable form of neuronal ...mage. These neuronal alterations are found main... in areas in the brain which seem to exhibit selec... ...bility to oxygen deprivation (Brierly et ...

...efore cell death occur in the brain, ...ation initiates rapid and profound alt...abo... changes in ...ia, anoxia or ischemia have been extensively studied in the adult (Siesjö & Nilsson 1971 Siesjo & Plum 1971) and the neonatal animal (Jilek 1970, Vannucci & Duffy 1976) Before changes in oxidative and carbohydrate metabolism are detected

Table 1 *Tyrosine levels (µg/g wet weight) in various rat brain regions at different ages during normoxia and hypoxia*

Shown are mean ±S E. Figures within parentheses indicate the number of experiments. Comparisons by t-test. n.s. not significant

Age	Hemispheres	Striatum	Midbrain	Brain stem
4 days				
Control	‿8 6±1 58 (9) n s	32.6±1 93 (9) n.s	34 4±5 06 (5) n.s.	3‿8± 01 (8) n.s.
Hypoxia	‿6 7±1 9? (9)	?9.5± ?6 (9)	31 9±2.33 (7)	28.1± 01 (9)
14 days				
Control	?8 ?±2.13 (10) n s	4 ±2.51 (10) n s.	‿3 9±1 73 (10) n.s.	30.7±3 46 (10) P 38
Hypoxia	‿3 9±2.35 (10)	4.5±1 98 (10)	1‿±1 61 (10)	‿ 6±1.36 (10)
28 days				
Control	?4 4±1 46 (12) n s	18.4±1 63 (10) n s.	4 4±1.39 (9) n s.	‿3±2 09 (11) n.s.
Hypoxia	18.5±1.57 (11)	15 8±1.24 (11)	?0.5±1 65 (9)	18.3±1 81 (11)

the infant rats were exposed to a N_2–O_2 environment in a sealed ‿‿-litre plastic cage for 30 min. All experiments started around 4 h after the onset of light The oxygen content of the gas mixture was 6% and the mixture was let through the cage via inlet and outlet holes at a rate of about 4 liter/min Control animals were kept in a similar open box exposed to room air The cages were kept on a preheated (35–36°C) table and the room temperature was ??°C

All animals were injected subcutaneously with NSD 1015 100 mg/kg (3-hydroxybenzylhydrazine HCl synthetized in this laboratory by Dr P Lindberg) 30 min before sacrifice The infant rats were killed by decapitation immediately after 30 min exposure to the gas mixture The whole brain (without olfactory lobes) was quickly removed and dissected on an ice-cold glass plate into the following parts. (1) Striatum including corpus striatum and limbic forebrain ( ) hemispheres including hippocampus, (3) diencephalon ("midbrain) and (4) lower brain stem. Cerebellum was identified and discarded DA is the predominating catecholamine in part (1) and NA in parts ( ) (3) and (4) Most of the 5-HT containing nerve cell bodies are found in part (4) For details of the dissection procedure see Carlsson & Lindqvist (1973).

Immediately after the dissection procedure the brain parts were frozen on dry ice In the 4 days old rats [illegible] were pooled in the 14 days old 4 parts were pooled and the ‿8 days old parts were pooled and weighed. The brain samples were stored in a freezer at 70°C, in no case for more than months.

After thawing, the pooled brain parts were homogenized in 10 ml of 0 4 N perchloric acid containing 5 mg Na_2SO_3 and ?0 mg EDTA The homogenates were centrifuged at about 10000×g for 10 min at 0°C and the supernatant purified on a strong cation exchange column (Dowex 50 X-4) (Atack & Magnusson 1978) The separated amines and precursors were analyzed spectrofluorometrically according to previously described techniques (see And 1977)

Statistical analysis was performed using Student's t-test P values larger than 0 05 were considered not significant

Table *Tryptophan levels (µg/g wet weight) in various rat brain regions at different ages during normoxia and hypoxia*

Shown are mean ±S.E Figures within parentheses indicate the number of experiments Comparisons by t-test n.s. not significant

Age	Hemispheres	Striatum	Midbrain	Brain stem
4 days				
Control	6.0±0.43 (9) n s.	6 5±0 46 (9) n	6 7±0 49 (9) n s	6 2±0.40 (9) n s.
Hypoxia	6.6±0 54 (9)	7 6±0 65 (9)	7 4±0 59 (9)	7 1±0 68 (9)
14 days				
Control	7 0±0 49 (10) n s	7 2±0 32 (10) n	5 1±0.59 (10) n s	6.5±0.53 (10) n.s
Hypoxia	7 0±0.31 (10)	6 9±0 21 (10)	5 1±0.33 (10)	6.4±0 26 (10)
‿8 days				
Control	5.5±0.?0 (12) n.s.	5 1±0 ‿5 (12) n s	5 5±0.‿8 (12) n	5.5±0 3 (1) n.s.
Hyoxia	5 6±0.33 (1?)	5 9±0.35 (12)	5.5±0.27 (12)	5 1±0.29 (1)

RESULTS

Tyrosine and tryptophan levels

Tyrosine and tryptophan levels tended to decrease in the various brain areas with advancing age. Exposure to hypoxia did not significantly alter the tyrosine levels in the different brain parts except a decrease noted in the brain stem at 14 days of age (Table 1). During 30 min of exposure to 6% O_2–94% N_2, no alteration in tryptophan levels was noted compared to controls (Table 2).

Dopa and 5-HTP accumulation after NSD 1015

Dopa accumulation after NSD 1015 in the various brain parts increased significantly over the developmental period studied (Fig. 1).

In the 4 days old animals there were significant decreases in Dopa accumulation during 30 min 6% hypoxia in all brain regions studied except for the hemispheres (Fig. 1). At 14 days of postnatal age hypoxia caused a significant decrease in Dopa accumulation after NSD 1015 in the hemispheres and brain stem (Fig. 1) and in the 28 days old animals a marked decrease was noted in all brain regions (Fig. 1).

An increase in 5-HTP accumulation after NSD 1015 administration was noted in the various brain parts with increasing age (Fig. 2).

During the 30 min exposure to 6% hypoxia, a marked decrease in 5-HTP accumulation was noted in all brain regions (hemispheres, striatum, medulla and brain stem) at all ages studied (Fig. 2). The relative decrease in the different brain parts appeared to be similar for the various ages studied.

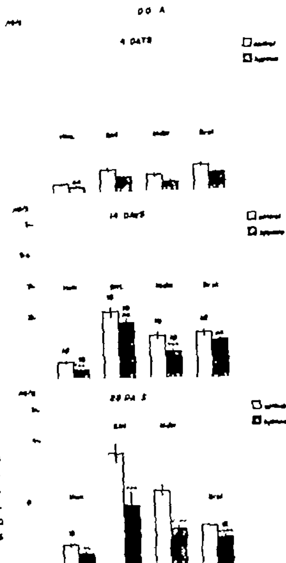


Fig. 1. Brain tyrosine hydroxylase activity in 4, 14 and 28 days old rats during hypoxia. Tyrosine hydroxylase activity was measured in various brain regions (*Hem*: hemispheres, *Str*: striatum, *Midbr*: midbrain, *Br.st*: brainstem) by means of Dopa-accumulation after inhibition of aromatic L-amino acid decarboxylase with NSD 1015 injected subcutaneously 30 min before sacrifice. Shown are means ±S.E. Figures indicate the number of observations. Comparisons by *t*-test. $*P<0.05$, $**P<0.01$, $***P<0.005$, $****P<0.001$, n.s. = not significant.

DISCUSSION

Experimental animal studies have shown that different forms of severe oxygen deprivation, such as hypoxia, anoxia or ischemia, have one common neuropathological denominator (Brierly et al. 1973) resulting in a highly predictable form of neuronal damage. These neuronal alterations are found mainly in areas in the brain which seem to exhibit selective vulnerability to oxygen deprivation (Brierly et al. 1973).

However, before cell death occur in the brain, oxygen deprivation initiates rapid and profound alterations in metabolic activity. The changes in oxidative reactions during hypoxia, anoxia or ischemia have been extensively studied in the adult (Siesjö & Nilsson 1971, Siesjö & Plum 1971) and the neonatal animal (Jilek 1970, Vannucci & Duffy 1976). Before changes in oxidative and carbohydrate metabolism are detected in the brain

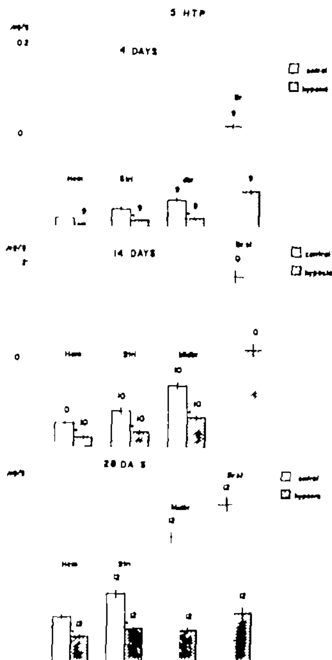


Fig 2 Brain tryptophan hydroxylase activity in 4 14 and 28 days old rats during hypoxia Tryptophan hydroxylase activity was measured in various brain regions (*Hem* hemispheres *Str* striatum *Midbr* midbrain *B* *t* brainstem) by means of 5-HTP-accumulation after inhibition of aromatic L-amino acid decarboxylase with NSD 1015 injected subcutaneously 30 min before sacrifice Shown are means ±S E Figures indicate the number of observation Comparisons by *t*-test $P<0.005$ $P<0.001$ n s =not significant

alterations in the synthesis and degradation of the monoamine neurotransmitters DA NA and 5 HT occur (Davis & Carlsson 1973 Hedner et al 1977*a* Hedner et al 1977*b*)

In the present study we have used NSD 1015 which is a potent inhibitor of the second enzyme both the catecholamine and the indoleamine synthetic pathways to measure tyrosine hydroxylase and tryptophan hyroxylase activity This can be achieved as the intermediate amino acids Dopa and 5-HTP respectively accumulate in a linear manner during the first 30 min after injection (Carlsson et al 1972)

During 30 min hypoxia there were, generally significant decreases in tyrosine hydroxylase activity in the different brain areas in the neonatal as well as in the 4 weeks old animals. Concerning 5-HT no apparent differences in sensitivity to hypoxia between the different neuronal populations were evident The inhibition of tryptophan hydroxylase was extensive in all brain areas and the relative decrease appeared to be similar for the various ages studied The decrease in Dopa accumulation appeared to be more pronounced in the striatum at 28 days (47%) than at 14 (n.s) and 4 days (32 6) of postnatal age One possible explanation for this discrepancy could be that monoamine neurons (mainly DA in striatum) are more metabolically active in older animals and therefore more sensitive to hypoxia

However the response of tryptophan hydroxylase to hypoxia did not seem to be correlated to the outgrowth of the serotonin neurons or other maturational events occurring in the rat brain during development such as e g capillary proliferation

The levels of hypoxia used in the present study are not likely to produce permanent neuronal damage in the rat brain However as a more severe oxygen deprivation causes permanent changes in brain monoamine metabolism (Hedner et al., to be publ) these mechanisms may possibly be relevant factors in certain childhood behavioral disorders

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nal proximal tubular acidification Role of brush-border
d cytoplasmic carbonic anhydrase

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KARLMARK, B., ÅGERUP B & WISTRAND P J Renal proximal tubular acidification. Role of brush-border and cytoplasmic carbonic anhydrase. Acta Physiol Scand 1979 106, 145-150 Received 14 Oct 1976. ISSN 0001-6772. Department of Physiology and Medical Biophysics and the Department of Medical Pharmacology Biomedical Center University of Uppsala, Sweden.

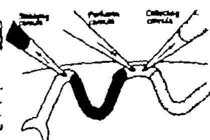
Carbonic anhydrase is found in the cytoplasm and brush border membranes of renal *proximal tubular cells Both the soluble and the membrane-bound enzyme ha e been* assigned roles for the secretion of hydrogen ions into the tubular fluid and hence also for the reabsorption of bicarbonate. Attempts were made to differentiate bet een the roles of these enzymes for the rate of proximal tubular acidification Proximal tubules of rats were instilled and perfused with bicarbonate solutions containing carbonic anhydrase inhibitors, especially designed to be impermeable to cell membranes. The acidification rate was measured with an antimony micro-electrode ystem—the only instantly responding micro-pH electrode The membrane impermeable inhibitors had no effect on this ate in contrast to acetazolamide which markedly inhibited the acidification rat when administered intraluminally It is therefor concluded that the cytoplasmic carbonic anhydrase is the important enzyme for the proximal tubular acidification ate, and hence the ate of bicarbonate *reabsorption The function of the brush border enzyme remain an outstanding* problem

Key ords Carbonic anhydrase carbonic anhydrase inhibition, acetazolamide, urine acidification, hydrogen ion secretion, CO_2-transfer bicarbonate reabsorption, proximal tubule, brush border antimony micro-electrodes

physiological role of carbonic anhydrase (car *ate hydrolyase E c 4.2.1 1)* has been studied ly by the use of inhibitors. It led Pitts & Alex er (1945) to make the proposition that the renal yme participates *the reabsorption of bicarbo-* e ions from the renal tubule The enzyme was ught of as being located intracellularly and in- ved in the mechanism of secretion *of hydrogen* s from the proximal and distal tubular cells A oplasmic enzyme from kidneys has since been racterized in detail in man (Wistrand et al 1975) *d* in rats (Wistrand & Wåhlstrand 1977) These dies showed that there appears to be only one m of soluble enzyme in the kidneys, identical *th the* high-activity form (with respect to hydra- n of CO_2) found in the erythrocytes.

Berliner (1957) and Walser & Mudge (1960) sug- sted that there existed also a luminally active carbonic anhydrase Data from micropuncture studies (Rector et al 1965 Vieira & Malnic 1968) were interpreted to support this prediction Recent histochemical (Lonnerholm 1971 Lönnerholm & *Ridderstråle 1979)* and biochemical studies (Wistrand & Kinne 1977) have shown that carbonic anhydrase acti ity is indeed closely associated with the brush border *membranes of the proximal tubu-* lar cells of the rat kidney The activity originates from an enzyme probably different from the cyto- plasmic form (Wistrand 1979) *Rector et al (1965)* proposed that the activity of this membrane-bound enzyme would be to prevent the accumulation of carbonic acid in the proximal lumen and hence facilitate the hydrogen ion secretion by reducing the pH-gradient between the cell interior and the tubular fluid.

Thus, the proximal tubules contain carbonic an-



The arrangement of the cannulas during the perfusion experiments. The stabilizing cannula collects the ...al urine flow. The collecting cannula collects all the ...ate by balancing the distal (dotted) oil block.

...standard procedures. The left kidney was exposed ...flank incision and fixed in a lucite cup using % ...agar in saline. After the gelatinization, small cap ...removed from the agar-agar leaving a small hole ...was filled with mineral oil. The urine delivered to ...bladder was drained to prevent a drop in GFR due to ...bladder tension (Kuntz et al. 1973). The blood pressure was continuously checked, and no further expts ...performed if the blood pressure at any moment became lower than 100 mmHg. The fluid lost during the ...was compensated for by infusing rat Ringer solution ...rate of 4 ml kg⁻¹ h⁻¹.

...e blood acid-base status was measured with the conventional Astrup technique and using the nomogram ...used for man.

...tratubular pH-measurements were made using the ...mony microelectrode. The manufacturing and use of ...are described elsewhere (Karlmark 1973). The reference electrode was an Ag/AgCl-electrode connected to ...ground tip of the tail of the rat.

...our hands the electrode gives linear calibration in ...filtrate-like buffer solutions with a pH below 7.6, but ...the pH about 0.16 pH-units too low in the ...physiological bicarbonate range.

...e antimony electrode as pH-monitor has been debated extensively in recent years (cf. Caflisch et al. 1978) ...experts agree however that if the calibration of the ...ode occurs in solution such is similar to that of ...unknown, the electrode works well for micropuncture ...important in this context is also that the antimony ...electrode gives an immediate response, which is a ...ssity when studying systems where chemical nonequilibrium exists like in the proximal tubular lumen after ...bonic anhydrase inhibition (Rector et al. 1965).

...he determination of bicarbonate was performed in 5 nl ...ples using the equilibration technique as described by ...mark & Sohtell (1973). The buffer capacity of the ...ples was supposed to be low enough to permit calculation according to the "mean value method" (Karlmark & ...tell 1973). The bicarbonate concentration was regarded to be unaffected by the difference in P_{CO_2}.

...stillation experiments. This experimental series was ...formed using the antimony instillation technique with pH-measurements as described by Malnic & de Mello-Aires (1971). One column of mineral oil, introduced into the proximal tubule was split up by the test solution (see below). The antimony micro-electrode previously introduced in the same loop adjacent to the instillation cannula, started to record the intratubular pH in the droplet. Due to the secretion of hydrogen ions and/or the direct reabsorption of bicarbonate ions, the pH declined in an exponential-like manner.

The instillation solutions contained 150 mM sodium bicarbonate with or without inhibitor against the brush border-bound carbonic anhydrase. The inhibitor concentrations were chosen to be at least 100 times the I_{50} (Table 1) i.e. 6 μM for acetazolamide, 0.1 mg/ml for the dextran-coupled sulfonamide (Dy 97 A) and 0.1 mM for the quaternary sulfonamide (QAAS).

Perfusion experiments. In this experimental series, microperfusion expts. on proximal tubules of 11 rats were made. Fig. 2 shows the technique schematically. The perfusion time was 4–5 min, with luminal perfusion rate of 15 nl/min. An equilibrium solution containing 80 mM raffinose, 25 mM $NaHCO_3$, 85 mM NaCl, 3 mM KH_2PO_4, 1 mM K_2HPO_4, with or without inhibitor was used for the perfusions. The concentrations of the inhibitors were 6 μM for acetazolamide and 0.1 mg/ml of Dy 97 A. In these expts. bicarbonate was measured instead of pH, since the distances in the tubule between the perfusion cannula and the site of measurement, was too short to permit simultaneous measurements of pH and bicarbonate without introducing considerable error. The perfusion solutions were equilibrated with PCO_2 of 40 mmHg in wet gas for 1 h. The equilibration apparatus was constructed to permit filling of the perfusion cannula without hypobaric pressure, which releases the gas dissolved. A constant PCO_2 of the perfusion solution was maintained in this way.

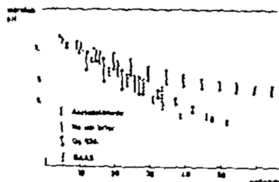


Fig. 3. Instillation experiments. The rate of decrease in intratubular pH after intraluminal administration of different carbonic anhydrase inhibitors. The total variations of pH in the instillation solutions are given at the different times. Dy 97 A and QAAS refers to the membrane impermeable carbonic anhydrase inhibitors, described in the Methods section.

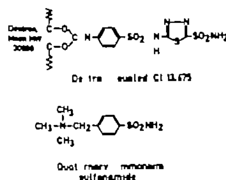


Fig 1 Structures of the membrane impermeable carbonic anhydrase inhibitors

hydrase as one soluble form localized in the cytoplasm and one membrane-bound form in the brush borders both thought to be involved in the secretion of hydrogen ions and in the reabsorption of bicarbonate

So far it has been difficult to test the function of each form since available inhibitors used as tools for this purpose will inactivate them both In the present work an attempt was made to dissociate the physiological role of the enzyme for the acidification process after selective inhibition of only the luminal enzyme by a new type of inhibitors

MATERIAL AND METHODS

Membrane impermeable inhibitors

CI 13475 (Fig 1) was chosen for the coupling to the dextran molecules, because of its strong inhibitory activity (K_i = nM) its length (~15 A) and its amino-group on the benzene ring which allows covalent binding of the sulfonamide to the bromcyanide activated polysaccharide. The coupling was done according to the method used for soluble dextrans described by Kågedal & Åkerström (1971) The free sulfonamide was removed by ultrafiltration using an Amicon® UM 2 membrane and by gel filtration. Determination of the molecular weight distribution of the complex showed that about 50% of the molecules had a molecular weight of less than 17000. Sulphur analysis showed that 250 µmol of CI 13475 were substituted per g dextran, corresponding to about 8 bound molecules of CI 13475 per molecule of dextran The complex called Dy 97A was freeze-dried and stored in lyophilized form

Since the dextran molecules might be too large to reach all enzymic sites of the brush border it was thought desirable to construct a smaller inhibitor A quarternary ammonium analogue of α-amino-p-toluenesulfonamide (m p 48°C) called QAAS was therefore synthetized (Fig. 1) We assume here that this substance cannot enter the interior of the tubular cells by diffusion from the luminal side, or by some unknown system for base transport.

The activities of the inhibitors towards the rat carbonic anhydrases were tested by an electrometric d... pH-method (Wistrand & Kinne 1977). Details of the method are given in the legend of Table 1

Enzymes

Renal soluble carbonic anhydrase was isolated by ... chromatography of homogenates of the renal cortex ... 200 g Wistar rats as described by Wistrand & ... (1977) About 0.4 mg of a highly purified enzyme obtained per g wet cortical tissue Its specific activity 20 units µg protein Brush border membranes were isolated from the cortex of the kidneys of the same type rats The isolation procedure involved differential centrifugation followed by preparative continuous ... flow electrophoresis according to the method of Heidrich et al. (1972). About 10 mg of membrane protein was extracted from 1 g of wet cortical tissue. The membranes were homogenous and free from other cell organelles judged from marker enzyme enrichment and electron microscopic inspection (Wistrand & Kinne 1977) The ... contained carbonic anhydrase activity of between ... units mg protein This enzyme is an integral part of ... membrane and a separate protein compared to the cytoplasmic form (Wistrand 1979)

Micropuncture techniques

White rats weighing between 200–280 g were used ... experiments They were fed on commercial pellet ... water *ad libitum* until the start of the experiment Anesthesia was maintained with Inactin® 120 mg/kg ... in a 5% solution given intraperitoneally The temperature was kept constant at 37.5°C using a servo-controlled ... vice Tracheostomia and catheterisation of the right common carotid artery and the left jugular vein was performed

Table 1 *Inhibition of cytoplasmic and brush border-bound carbonic anhydrases of rat kidney ...*

Electrometric changing pH-method. The assay ... contained 10 mM sodium-phosphate buffer ... Na_2SO 500 µM EDTA bovine plasma albumin ... mg/ml I=0 135 initial pH=7 0, 0°±0.1 [CO_2] ... mM Contact time without CO_2 between inhibitor ... enzyme was 4 min. Uninhibited enzyme activity ... tween 1 5 and 0 9 units. One unit of carbonic anhydrase ... the amount of enzyme that doubles the rate of hydration ... CO_2. I_{50} is the concentration of inhibitor that reduces ... standard enzyme activity by half

Inhibitor	Enzyme inhibition (I_{50}) Brush border enzyme	Cytoplasmic enzyme
Dextran coupled CI 13 475 (Dy 97 A)	0.5 mg/l	0.47 ...
Quarternary ammonium sulfonamide (QAAS)	1 8 µM	0.2 µ...
CI 13 475	6 nM	2 nM
Acetazolamide	170 nM	70 nM

-ate would not be expected to reach the in- tular enzyme because of the short time of con- etween inhibitor and tubular cells during the sion. However acetazolamide should have s to and be able to inhibit the brush border ne even during the short contact times in this of expts. The lack of effect (Fig. 4) therefore s to the unimportance of the brush border ne for the bicarbonate reabsorption

-ken together the results from both types of puncture studies indicate that the brush bor nzyme is not involved in the process of acidifi- n in the proximal tubular lumen It can be ed of course that this enzyme was not inhi- in our expts due to inability of the inhibitors ach and bind to all enzymic sites on the brush er membrane This possibility cannot be ruled but seems unlikely since the same lack of ef as seen regardless of the size structure and ge of the inhibitors used Other ideas about the ion of the luminal enzyme should therefore be itiated. It has been suggested that carbonic drase facilitates diffusion of carbon dioxide ss cellular membranes (Enns 1967 Gutknecht 1977) Inhibition of membrane-bound enzyme d therefore reduce the diffusion rate of carbon ide from the tubular lumen through the tubular This could explain the disequilibrium pH ob- ed in the proximal tubular fluid by Rector et al 5) and Vieira & Malnic (1968) after carbonic ydrase inhibition. The problem of whether or this pH-disequilibrium depends on the activity he luminal enzyme could perhaps be solved by ctly measuring the CO_2 of the tubular fluid, after administration intraluminally of the present ydrase impermeable inhibitors By using CO_2 sitive microelectrodes Sohtell (1979) could w that P_{CO_2} rises in the proximal tubule after temic administration of acetazolamide

are grateful to the American Cyanamid Co Pearl er N Y U S A for the gift of Cl 13 475 and acetolamide to Pharmacia Co Uppsala, Sweden (Drs Lindberg and L Kågedal) for the synthesis of the ran-coupled Cl 13 475 and to Ferrosan A/S Malmö, Denmark (Drs P S ... and O ... Hansen) for the synthesis of the quarternized ...amide Brush border membranes ... ere kindly pro- ed by Dr R Kinne, Max-Planck Institut für Biophysik ...nkfurt/Main, Germany

The work as supported by the Swedish Medical Re- arch Council, grants no 2674 and 4253 the Bergvall ...ndation and the Medical Faculty of Uppsala Uni-

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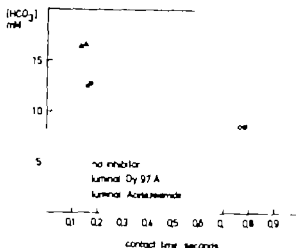


Fig 4 Perfusion experiments The decrease of bicarbonate concentration in the perfusate at different contact times after intraluminal administration of carbonic anhydrase inhibitors Dy 97 A is a dextran-coupled carbonic anhydrase inhibitor

RESULTS

Effects of membrane impermeable inhibitors on the enzymes in vitro

The inhibitory activity expressed as I_{50}, for the dextran coupled inhibitor CI 13475 was about 0 5 mg/l against the brush border enzyme (Table 1) This corresponds to 125 nM of bound CI 13475 Since the I_{50} of the free inhibitor was 6 nM only about 5% of the coupled sulfonamides appeared inhibitory The question arises whether this inhibition could be due to small amounts of free inhibitor contaminating the complex or liberated from it by hydrolysis of the covalent bond (cf Vauquelin et al 1975) We consider both possibilities unlikely since the coupled complex had been thoroughly filtered and was made up freshly from the freeze dried form just before use The other membrane impermeable inhibitor the quarternary sulfonamide was also active against both the brush border bound and the cytoplasmic form of the enzyme

Effects of the inhibitors in the micropuncture experiments

Control of blood pH and bicarbonate showed that the experimental procedures did not appreciably affect the acid-base status of the animals The results from the instillation expts are given in Fig 3 At time zero registration of the intratubular pH in the installation droplets started at an electrode potential of −400 mV pH 7.5 The figure shows the variation in pH as measured in the different hypo solutions instilled Of the inhibitors administered intraluminally only acetazolamide was able to diminish the rate of fall in pH

The data from the perfusion expts are presented in Fig. 4 This figure shows that in less than ... second about 50 of the bicarbonate perfused ... cond/about 50% of the bicarbonate perfused is affected by the membrane impermeable inhibitors or by acetazolamide added to the perfusate.

DISCUSSION

The purpose of the instillation expts was to ... a maximum hydrogen ion secretion from the proximal tubule Acetazolamide was found to markedly reduce the rate of this secretion (Fig. 3). This agrees with previous micropuncture studies in proximal tubules in the dog (Bernstein & C... 1968) rat (Clapp et al 1963 Rector et al ... Vieira & Malnic 1968 Kunau 1972) and the ... monkey (Bennett et al 1968) where carbonic anhydrase inhibitors were administered intraluminally or systemically

Acetazolamide is a weak acid (pK =7 4) and ... soluble at neutral pH It easily penetrates cell membranes as indicated by the high first order constant of 0 4 s^{-1} for its diffusion from plasma into red cells (Holder & Hayes 1965) Besides inhibiting the membrane-bound enzyme it ... therefore be expected to diffuse into the tubular ... and also inhibit the cytoplasmic enzyme during ... course of the instillation experiments. However the membrane impermeable inhibitors, especially manufactured to remain in the tubular fluid, should inhibit only the membrane bound enzyme. The enzyme of the brush border fragments is rapidly inhibited (as tested within 10 sec) by these inhibitors in vitro It should therefore be inhibited also in the instillation expts and by more than 99% ... the concentration in the luminal fluid was more than 100 times larger than the concentration found to inhibit the enzyme by 50% (I_{50}, Table 1). The lack of effect of these inhibitors would therefore indicate that the brush border enzyme is not involved in the acidification process Since acetazolamide inhibits both the cytoplasmic and the brush border enzyme it would therefore appear that its effect on acidification can be explained by its inhibition of the cytoplasmic enzyme

This is also borne out by the results of the perfusion expts In these expts, acetazolamide ...

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~mperature compensation of sodium transport and ·Pase activity in frog skin

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Na transport across frog skin, measured as short-circuit current (SCC) shows perfect temperature compensation in frogs acclimated to 6° 12° and 23°C as SCC values observed at the acclimation temperatures are equal (about 13 μA/cm²). Reacclimation experiments show that this is not a starvation effect. While very little temperature compensation is seen in the activity of Na K ATPase in epidermal homogenates from frog skins, the activity of Mg^{2+} ATPase shows inverse compensation at assay temperatures from 4° to 48°C. This ATPase is apparently activated either by Mg^{2+} or by Ca^{2+} and it probably controls the passive permeability of epidermal cells. It is suggested that the inverse temperature compensation in the activity of this enzyme is the main mechanism by which the observed perfect temperature compensation of Na transport across frog skin occurs.

Key words Temperature compensation, acclimation, sodium transport, ATPases, permeability frog, *Rana temporaria*

tive sodium transport is a basic function of animal cells. It establishes and maintains Na and K gradients across the cell membrane and is, in addition, an important mechanism of cellular thermogenesis (Edelman 1976). In many excitable cells, transport also continuously contributes to the resting membrane potential (Kerkut & York 1971 ...oms 1977).

Very little is known about the adaptation of Na transport to variations in environmental temperature. However the activity of the electrogenic Na pump seems to show compensatory acclimation to temperature in snail neurons (Merickel & Kater 1974).

Frog skin is a well-known system where the active transport of Na maintains a potential difference across the skin. Net Na flux across the skin is directly related to the current needed to bring the potential difference between the sides of the skin to zero (short-circuit current or SCC) (Ussing & Zerahn 1951). The temperature dependence of SCC in relation to the Na transport has been studied by Takenaka (1963). SCC is equal to the net Na transport at least between 6 and 20°C. On the other hand, the activity of the membrane-bound Na and K-activated Mg^{2+}-dependent adenosine triphosphatase (Na K ATPase) has been related to cation transport in frog skin (Bonting & Caravaggio 1963) as well as in other tissues.

As many functions of frogs show temperature compensation (Lagerspetz 1977*b*) it was decided to determine, whether SCC and the activities of the membrane-bound ATPases in the frog skin could show physiological adaptations to environmental temperature.

Kawada, Taylor & Barker (1975) have recently *directed attention to this problem.* In their experiments the SCC of skin preparations from *Rana pipiens* did not differ at 10° and 24°C and the previous acclimation of the animals to these temperatures was without effect. Our results are in contrast to these and suggest that Na transport across the frog skin shows unusually good temperature compensation.

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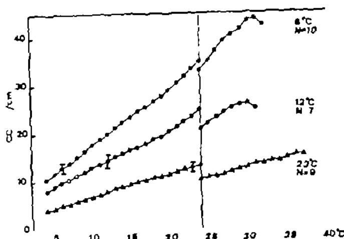


Fig. 1. Temperature dependence of the SCC of isolated ventral skins from frogs acclimated to 6°C (black dots), 12°C (circles) or 23°C (triangles). Number of test animals (N) is indicated at each graph. The left part of the figure gives averages of the SCC values measured during the lowering of the temperature of the chamber from 24° to 4°C and during the subsequent elevation of the temperature back to 24°C. The right part is based on values measured during the increase of temperature above 24°C. Vertical bars at the acclimation temperatures give ±S.E.

[illegible] of reacclimation on SCC

[illegible]n poikilothermic animals, like frogs, are transferred to higher environmental temperatures and [not] fed, it is possible that the effects observed [are due] to more profound starvation and thus on [lac]k of energy substrates in the animals kept at the [high]er temperature. Therefore 4 frogs were first [acclim]ated at 23°C for 2 weeks and then trans[ferre]d back to 6°C for reacclimation. After a period [of 2] weeks at 6°C they were used for the measure[men]t of SCC. The measurements were performed [at 6°, 1]2° and 23°C. The mean SCC values of these re[accli]mated frogs did not differ significantly at any [tem]perature from values recorded from animals [kept] continuously at 6°C (Table [2]). Starvation caused by a stay of 2 weeks at 23°C cannot therefore account for the lower SCC found in the 23°C acclimated group.

Effect of Na concentration on SCC in cold- and warm-acclimated frogs

The dependence of SCC on the Na concentration in the medium was studied in isolated ventral skins of frogs acclimated to 10° or 24°C for 1½ to 3 weeks using choline as the substitute for Na as described. The mean stabilized SCC values for skin preparations from 5 cold-acclimated and 5 warm-acclimated frogs at 7 different Na concentrations are given in Fig. 2. The previous thermal history of these ani-

[Tab]le 1. *Maximum SCC of the frog skin and the [temp]erature at which it was measured*

[Mea]n values for frogs acclimated to 6°, 12° or 23°C. [N de]notes the number of experimental animals.

Acclimation temperature (°C)	Maximum SCC (µA/cm²)	Temperature for maximum SCC (°C)	N
[illegible]	44±4	30.8±1.0	10
[illegible]	25±4	29.6±0.3	7
[illegible]	16 2	35.3±0.9	9

Table 2. *Mean SCC of skins from frogs acclimated to 6°C (10 animals) and from frogs acclimated first to 23°C and subsequently to 6°C (the reacclimated group, 4 animals) at three measurement temperatures*

Measurement temperature (°C)	Mean SCC (µA/cm²) Acclimated to 6°C	Reacclimated to 6°C
6	13±1	11±1
12	19±2	17±1
23	33±2	33±1

MATERIAL AND METHODS

Frogs (*Rana temporaria*) of either sex were collected in late autumn from their local wintering places in SW Finland and stored at 6°C (±1°C) in basins with shallow running tap water Some animals were transferred to containers at 12°C (±1°C) or 23°C (±1°C) for periods indicated in the Results section. The animals were not fed

The SCC of the ventral skin was measured in a lucite double chamber (Ussing & Zerahn 1951) with an exposed skin surface area of 3 1 cm² Bot sides of the chamber usually contained about ?0 ml of continuously aerated Ringer solution (115 mM NaCl KHCO 2.5 mM 1 mM CaCl) For temperature experiments the chamber was kept in a thermocontrolled water bath The experimental temperatures were measured with a thermocouple close to the skin in the chamber An equilibration time of at least 1 h was allowed before the onset of SCC measurements.

The open-circuit potential across the skin was monitored by agar KCl bridges in close proximity to both sides of the skin and connected via calomel electrodes to a digital millivoltmeter The skin was short-circuited through Ag AgCl electrodes in both ends of the chamber They were connected to a D C source through a potentiometer and a microammeter which was used for the measurement of the SCC

In the experiments on the effect of temperature on SCC the chamber was first cooled from 4° to 4°C with SCC readings taken for each decline of a full degree Before each reading, a stabilization period of –3 min was allowed Subsequently the chamber was heated from 4° to 31–40°C with SCC readings being taken for each elevation of a full degree For every skin studied between 4° and 4°C the SCC values were thus recorded both during the decrease and the elevation of the experimental temperature However from 4° to higher temperatures readings were only taken during the rise since the highest temperatures used obviously damaged the skin

The dependence of SCC on the Na concentration in the medium was studied using Ringer solutions in which a varying part of the NaCl ontent was replaced by choline chloride The measurement temperature was about 23°C After the initial equilibration of SCC in normal Ringer (115 mM Na) the Ringer solutions on both sides of the skin were changed at intervals The other Na concentrations used were 93 70 48 25 14 and 7 mM A period of 10–20 min was sufficient for the stabilization of the SCC at each concentration.

For the ATPase activity determinations the epidermal sheets of 3 to 5 ventral skins (1 7 to 3 g fresh weight) were separated from the dermis by soaking the skins in cold 1 M NaI solution, which also contained 100 mM Tris-HCl buffer (pH 7 4) for 30 min (Kawada, Taylor & Barker 1969) The homogenization of the epidermal preparations and the ATPase assays were carried out as described by Kawada Taylor & Barker (1969 1975) The epidermal sheets were first washed in 5 mM EDTA made in 100 mM Tris-HCl buffer (pH 7 4) They were then homogenized with an all-glass homogenizer in 0 25 M sucrose solution made in the Tris-HCl buffer These 10–15% homogenates were centrifuged at 900 g in cold for 10 min and the supernatants were used for enzyme assays.

The incubation media routinely used consisted of 3 mM ATP as Tris salt 100 mM Tris-HCl buffer (pH and 6 mM $MgCl_2$ or of this solution with 100 mM Na and 10 mM KCl added. In some experiments different concentrations of $MgCl_2$ or $CaCl_2$ were used to also study the divalent cation requirements of the ATPase activity The assays were carried out with duplicate tubes each containing 0.5 ml of the supernatant in a total volume of ml After temperature equilibration, the supernatant was added and the tubes incubated for 20 min at 37°C In temperature dependence experiments, the tubes were incubated at different temperatures ranging from 4 to 49°C After incubation, inorganic phosphate liberated was determined (Atkinson Gatenby & Lowe 1973), and the protein content of the supernatants from perchloric acid precipitates dissolved in NaOH (Lowry et al 1951) The enzyme activities were calculated as micromoles inorganic phosphate liberated mg protein⁻¹ h⁻¹ and the activity of Na K ATPase determined as the difference in the enzyme activity in the presence of Na K and Mg² and in the presence of Mg² only

The results were analysed statistically using the t-test The deviations were expressed as standard errors of the mean (±S E)

RESULTS

Effects of temperature and temperature acclimation on SCC

The frogs used in these experiments had been acclimated to 6 12° or 23°C for ?–4 weeks, and the SCC was measured at different temperatures from 4 to 31–40°C as described Results are given in Fig 1 In each group the SCC was clearly temperature-dependent The mean Q values calculated for the temperature interval 4 –?4°C were 1.84, 1 ? and 1 84 for frogs acclimated to 6 12° and 23°C respectively However also the acclimation temperature affected the mean SCC the 6°C-group had the highest mean SCC values at each measurement temperature and the 23°C group the lowest. As can be seen in Fig 1 the SCC values measured at the respective acclimation temperature of each group did not differ significantly from each other and were all about 13 $\mu A/cm^2$ This indicates perfect temperature compensation of the SCC

As shown by the data given in Fig 1 and in Table 1 the upper range of the temperature dependence of the SCC was also changed by temperature acclimation The temperature for the maximum SCC was shifted upwards significantly ($P<0.01$) in the group acclimated to the highest temperature (23°C when compared with the t r groups

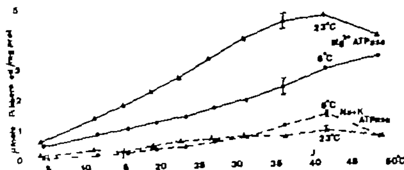


Fig. 4. Temperature dependence of the activities of Mg^{2+}-activated ATPase (—) and Na+K activated Mg^{2+}-dependent ATPase ( ) in 900 g supernatants of epidermal homogenates from frogs acclimated to 6°C (●) or 23°C (Δ). Points give the means from 4 separate determinations with animals acclimated to 6°C and from 3 determinations with those acclimated to 23°C. Epidermis from 3 to 5 ventral skins was pooled for each determination. Vertical bars indicate ± S.E.s at other points were of the same magnitude as those given in the figure.

...tion medium in order to chelate the small ...ants of Ca^{2+} probably present in the homogen... supernatants used for the enzyme activity deter...ations. This did not affect the activity of the ... ATPase. On the other hand the substitution ...Mg^{2+} by Ca^{2+} also induced ATPase activity ... effects of different concentrations of Mg^{2+} ... Ca^{2+} on the ATPase activity are given in Fig. 3 ...arently both divalent cations stimulated the ...Pase activity of epidermal homogenates.

...ects of temperature and temperature ...imation on the ATPase activities

...e activities of Na K ATPase and Mg^{2+} ...Pase were measured at different temperatures in ...dermal homogenates made from frogs accli...ted for about 2 weeks to 6° or 23°C. The results of ...se experiments are given in Fig. 4. Temperature ...imation did not much affect the activity of Na ...-ATPase. Cold acclimation increased significant... only the maximum activity of this enzyme meas...ed at 42°C. The effects on the Mg^{2+} ATPase ...tivity were more pronounced. At all assay tem...ratures, the activity of Mg^{2+} ATPase was higher ...al homogenates made from skins of frogs ... 23°C than in those of frogs kept at ...easured at 4°C the activity of Mg^{2+} ... about 100% higher in preparations ...ated than in those made ...als.

DISCUSSION

In our experiments, the SCC of the isolated ventral skin of the frog *Rana temporaria* was positively temperature-dependent between 4° and about 30°C. This result is in agreement with results reported earlier both for this species and for *Rana pipiens* (Francis 1933; Snell & Leeman 1957; Takenaka 1963; Dalton & Snart 1968).

An acclimation of frogs to 6°, 1?° or 23°C for a period of 10 days to 4 weeks caused a temperature compensation of the SCC. Reacclimation expts. in which the frogs were first acclimated to 23°C and subsequently to 6°C showed that this effect was not due to lack of energy substrates caused by a more profound starvation at 23°C. The temperature compensation found was perfect, i.e. the mean SCC values measured at each of the three acclimation temperatures were not statistically different from each other. This result is in contrast to the only previous report on the effect of temperature acclimation on SCC in frog skin (Kawada, Taylor & Barker 1975). In their expts. acclimation for 3 weeks at 10° or 24°C failed to affect the SCC in *Rana pipiens*. In addition their data, in contrast to those of other authors, failed to show a positive dependence of SCC on the measurement temperature. This and the large variability of the SCC values found for different preparations may point to some methodical difference. On the other hand it is possible that these discrepancies could depend on a seasonal dif-

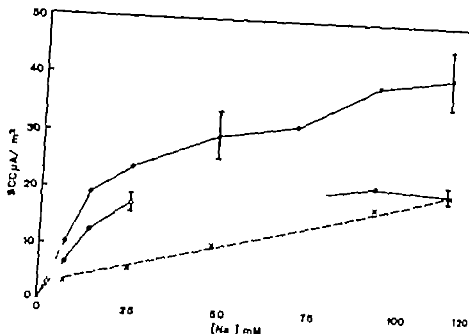


Fig 2 Effect of Na concentration on the SCC across the skins of frogs acclimated to 10°C (black dots) or 24°C (circles) Each point gives the mean from 5 experiments. Vertical bars indicate ± S E Crosses represent the differences of the mean SCC values found for the 10°C and the 24°C-acclimated groups. The lowest broken line is the calculated regression line for the differences.

mals again affected their SCC values In normal Ringer (115 mM Na) the mean SCC was 41 ± 5 µA/cm² in the skin preparations from cold-acclimated and 21 ± 2 µA/cm² for the warm-acclimated frogs respectively Values of SCC not statistically different from these were reached using choline-Ringer containing 48 mM Na by the group acclimated to 10°C and using Ringer containing 25 mM Na by the group acclimated to 24°C

While the shape of the relationship between Na concentration and SCC in the warm-acclimated animals was indicative of a saturable process only this relationship in the cold acclimated frogs could be best described by the sum of a linear and a saturable component. This became clear when the differences between the mean SCC values found for the cold-acclimated and the warm acclimated animals were plotted against Na concentration (Fig. 2) The linear correlation between the difference in the SCC and the Na concentration was high ($r = 0.99$)

ATPases of epidermal preparations

According to the studies of Kawada, Taylor & Barker (1969 1975) homogenates of the isolated epidermis of the frog ventral skin show two types of ATPase activity The ouabain sensitive Na K ATPase probably mediates the transport of Na across the inward-directed membranes of epidermal cells to the interstitial fluid (Bonting & Ca[illegible] vaggio 1963 Biber Chez & Curran 1966) The other ATPase which is ouabain-insensitive and Mg²⁺ activated shows a relatively high activity in epider[illegible] mal preparations but its function is so far uncle[illegible]

In our experiments the activities of these ATPAses were similar as those reported by Kawada Taylor & Barker (1969 1975) In 3 exp[illegible] 0 5 mM EGTA (ethyleneglycol-bis-(β-aminoethyl ether)-N N tetraacetic acid) was added to the [illegible]

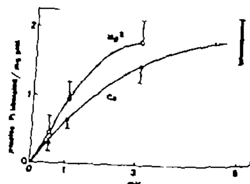


Fig 3 Effect of different concentrations of Mg² (circles) and Ca² (black dots) on the ATPase activity in 900 [illegible] supernatants of epidermal homogenates of frog skin. Incubation media contained also 3 mM Tris-ATP and [illegible] mM Tris-HCl buffer (pH 7 4) Incubation temperature was 37°C Each point gives the mean from 5 expts. and vertical bars indicate S.E.

ncrease in the passi e permeability to Na It f interest in this connection that the addition of er Mg² or Ca² to the outside medium of frog 1 decreases the net Na transport across the 1, probably by reducing the permeability of the er membranes to Na (Curran & Gill 196..)

f as suggested, Mg² ATPase controls the sive permeability of the cell membranes of frog dermis, then the inverse temperature compensa- 1 of its acti ity is physiologically adaptive An ation of temperature directly increases the flui- of lipid membranes and thus, their permea- ty An increase in the activity of Mg² ATPase ing arm acclimation could counteract this ect. Correspondingly a decrease of the activity this enzyme could oppose the immediate effect low temperatures. In erse compensation of the vity of Mg² ATPase would thus be adaptive for homeostasis of membrane permeability It ld function like the often described increase in proportion of saturated fatty acids in the mem- e phospholipids in a warm en ironment This nomenon has been called homeoviscous ptation of membranes (Sinensky 1974 Cossins rosser 1978)

s stated earlier the entrance of Na across the er epidermal membrane barrier probably is the -limiting step in the transport of this ion across frog skin (Cereijido et al. 1964 Ferreira et al 3 Ussing, Erl y & Lassen 1974). If the permea- ty of the membranes of epidermal cells to Na in part, controlled by Mg² ATPase and is refore changed during temperature acclimation compensatory way then the acclimation to high peratures would tend to decrease the permea- ity to Na and the net transport of Na across skin. The effects of cold acclimation would be opposite to these This could be the main chanism by which the observed perfect tempera- re compensation of SCC and consequently of Na transport across the frog skin occurs

e authors are indebted to Mrs Erika Holmbom and Ola Arpamaa, Mag. Phil for skilful technical assis- nce and to Mrs Sinikka Hillgren and Mrs Varpu ... Mag. Phil for the drawing of figures The ork as supported financially by the Academy of Finland.

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ference such as that found in the effect of antidiuretic hormone on SCC in *Rana temporaria* (Hong et al 1968)

In the expts on the effect of temperature acclimation on the dependence of SCC on Na concentration choline was used as the substitute for Na Since Kirschner (1955) reported that choline had no influence on the ionic flux it has been widely used as Na substitute in studies on the effect of Na concentration on Na transport across frog skin (Snell & Leeman 1957 Cereijido et al 1964) On the other hand Macey & Koblick (1963) found that choline ion itself had a stimulating effect on SCC However in the present expts choline was used to replace Na in both acclimation groups and the results can therefore be considered comparable with each other

The rate limiting step in the transport of Na across the frog skin is probably the entry of this ion across the outer epidermal barrier (Cereijido et al 1964 Ferreira Guerreiro & Ferreira 1973 Ussing Erlij & Lassen 1974) Our expts on the effect of Na concentration on SCC disclosed a linear component in this relationship in the case of cold acclimated animals but none such for the warm-acclimated frogs As the linear component is indicative of a diffusional process (Biber & Curran 1970 Ferreira et al 1973) cold acclimation probably increased the passive permeability of the outer membranes of epidermal cells to Na

Additional evidence about the mechanism of the temperature compensation of Na transport was gained from the ATPase activity studies When the activity of Na K ATPase of epidermal preparations was measured at different temperatures ranging from 4° to 48°C a significant difference between the enzyme activities in epidermal homogenates from frogs acclimated to 6 or 23°C was only found at the assay temperature of 47°C This is about 11 higher than the temperature for the maximum SCC in the skin of cold acclimated frogs It therefore seems unlikely that the temperature compensation described depends on modifications in the active Na transport mechanism

On the other hand over the entire temperature range studied remarkably large differences were found in the activities of the Mg^{2} ATPase from the epidermis of frogs acclimated to 6 or 23°C In this case the enzyme activity was higher in epidermal homogenates from warm-acclimated frogs The same effect was reported by Kawada Taylor & Barker (1975) The activity of Mg^{2} ATPase in the frog epidermis thus shows inve[rse] temperature compensation (Hazel & Prosser 197[4]) Frog skin is not the only tissue in which the accli[-] mation to higher temperatures increases the activ[ity] of this enzyme Similar effects have been found [in] frog brain microsomes (Lagerspetz 1977a), in the brain gills and intestine of goldfish (Kato 19[..] according to Hazel & Prosser 1970 Smith & Ellory 1971) and in the kidney microsomes of the c[..] (Tirri Vornanen & Cossins 1978)

Knowledge of the functions of Mg^{2} ATPase ap[-] pear essential for the interpretation of the inver[se] temperature compensation of its activity In our ex[-] periments chelation of the small amounts of end[o-] genous Ca^{2} did not affect the enzyme activity On the other hand ATPase activity was al[so] induced by concentrations of Ca^{2} comparable [to] the activating Mg^{2} concentrations. It is therefor[e] possible that the enzyme in question is activated by either divalent cation and is a Mg^{2} or Ca^{2} activated ATPase Similar ATPases maximal[ly] activated by relatively high (0 3–10 mM) concentra[-] tions of either Mg^{2} or Ca^{2} have been found [in] vertebrate brain synaptosomes and microso[mes] (Berl & Puszkin 1970 Saermark & Vilhardt 19[..] Trams & Lauter 1978) in the membranes of c[ul-] tured fibrocytes and neurones (Willingham, Ostl[..] & Pastan 1974 Lauter Duffard & Trams 1977), [as] well as in the gill microsomes of the fresh w[ater] mussel *Anodonta* (Senius & Lagerspetz 1978).

Bowler & Duncan (1967a 1967b Duncan 19[..]) have suggested that the function of the membra[ne] bound Mg^{2} ATPase is to control the passive [per-] meability of cell membranes perhaps acting [as a] contractile mechanoenzyme An increased act[ivity] of Mg^{2} ATPase would then tend to decrease passive permeability to ions and other dissol[ved] substances The results obtained in experim[ents] with the gills of fresh water mussels are in ag[ree-] ment with this concept (Senius and Lagers[petz] 1978) The Mg^{2} ATPase found in *Anodonta* microsomes seems to be activated also by C[a] and the same probably applies for the enzyme [in] frog epidermis Furthermore our results sug[gest] that the Mg^{2} ATPase found in the frog epide[rmis] may have also a permeability-controlling func[tion] Cold acclimation strongly decreases the activ[ity of] this enzyme and at the same time changes the [rela-] tionship between Na concentration in the me[dium] and SCC across the skin in a way which indi[cates]

ncrease in the passi e permeability to Na It interest in this connection that the addition of r Mg^{++} or Ca^{++} to the outside medium of frog decreases the net Na transport across the probably by reducing the permeability of the r membranes to Na (Curran & Gill 196_)
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ı effect of surgical sympathectomy and of neonatal ·atment with 6-hydroxydopamine and guanethidine ᴶparticle-bound noradrenaline and ³⁵-sulphomucopolysaccharides

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BLASCHKE, E. & UVNÄS B The effect of sympathectomy and of neonatal treatment with 6-hydroxydopamine and guanethidine on particle-bound noradrenaline and ³⁵S-sulphomucopolysaccharides Acta Physiol Scand 1979 106: 159–167 Received 17 Nov 1978. ISSN 0001-6772. Department of Pharmacology Karolinska Institutet, Stockholm, Sweden.

Five to six weeks after bilateral stellate ganglionectomy noradrenaline (NA) levels in cats' atria were reduced to ~20% of controls. In vivo uptake of ³H-NA and of ³⁵S-sulphate into gradient fractions containing noradrenergic vesicles from the atria decreased to ~30% and ~40% respectively The uptakes of ³H-NA and ³⁵S-sulphate were significantly correlated in both control and ganglionectomized cats, and the distributions of ³H and ³⁵S on the gradients were parallel The findings suggest that sulphomucopolysaccharides (SMPSs) may be localized in noradrenergic vesicles, possibly participating in the storage of NA. In 10-week-old rats treated neonatally with either 6-hydroxydopamine (6-OH-DA) or guanethidine, levels of NA in the heart, spleen and salivary glands were decreased to <10% and to 10–20% respectively in the seminal ducts to 33% and 45% respectively ³H-NA uptake into noradrenergic vesicle-enriched subcellular fractions from the heart, spleen and salivary glands of 6-OH DA treated rats decreased almost to the extent of NA depletion but in the seminal ducts the decrease was less marked. Guanethidine treatment left the uptake unaffected, except for the spleen. The discrepancy between storage and uptake suggests that surviving neurons display during their outgrowth into tissues high uptake capacity but lack full NA synthesis. ³⁵S-sulphate incorporation into non-lipid compounds, presumably SMPSs, in the noradrenergic-vesicle-enriched fractions appeared unaffected or increased over corresponding control levels, possibly due to high synthetic activity in the growing neurons

Key words: 6-hydroxydopamine guanethidine, noradrenaline, subcellular fractionation, sympathectomy

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[illegible]e effect of surgical sympathectomy and of neonatal [illegible]atment with 6-hydroxydopamine and guanethidine [illegible]'particle-bound noradrenaline and [illegible]-sulphomucopolysaccharides

[illegible]ONORA BLASCHKE and BORJE UVNAS
[illegible]tment of Pharmacology Karolinska Institutet, Stockholm, Sweden

BLASCHKE, E. & UVNÄS, B. The effect of sympathectomy and of neonatal treatment with 6-hydroxydopamine and guanethidine on particle-bound noradrenaline and ^{35}S-sulphomucopolysaccharides. Acta Physiol Scand 1979 106: 159–167 Received 17 Nov 1978. ISSN 0001-6772. Department of Pharmacology Karolinska Institutet, Stockholm, Sweden

Five to six weeks after bilateral stellate ganglionectomy noradrenaline (NA) levels in cats atria were reduced to ~20% of controls. In vivo uptake of ^{3}H-NA and of ^{35}S-sulphate into gradient fractions containing noradrenergic vesicles from the atria decreased to ~30% and ~40% respectively. The uptakes of ^{3}H-NA and ^{35}S-sulphate were significantly correlated in both control and ganglionectomized cats, and the distributions of ^{3}H and ^{35}S on the gradients were parallel. The findings suggest that sulphomucopolysaccharides (SMPSs) may be localized in noradrenergic vesicles, possibly participating in the storage of NA. In 10-week-old rats treated neonatally with either 6-hydroxydopamine (6-OH-DA) or guanethidine, levels of NA in the heart, spleen and salivary glands were decreased to <10% and to 10–20% respectively; in the seminal ducts to 33% and 45% respectively. ^{3}H-NA uptake into noradrenergic-vesicle-enriched subcellular fractions from the heart, spleen and salivary glands of 6-OH-DA treated rats decreased almost to the extent of NA depletion but in the seminal ducts the decrease was less marked. Guanethidine treatment left the uptake unaffected, except for the spleen. The discrepancy between storage and uptake suggests that surviving neurons display during their outgrowth into tissues high uptake capacity but lack full NA synthesis. ^{35}S-sulphate incorporation into non-lipid compounds, presumably SMPSs, in the noradrenergic-vesicle-enriched fractions appeared unaffected or increased over corresponding control levels, possibly due to high synthetic activity in the growing neurons.

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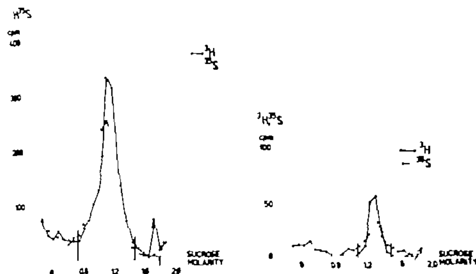
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a and *b* Distribution of H and ^{35}S radioactivity on continuous density gradient centrifugation of the $P_{...}$ pellet homogenized cat atria. Cats were injected with $Na_2{}^{35}SO$ (s.c.) and ^{3}H-NA (i.) 24 h and 1.5 h before sacrifice tively ( ) Control cat (right atrium); (*b*) bilaterally ganglionectomized cat (both atria)

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...SULTS

...ts of ganglionectomy (cats)

...terally ganglionectomized cats were sacrificed ...weeks after the removal of the right stellate ...glion (main supplier of noradrenergic fibres to ...atria, Goodall 1951) following a s.c. injection of $Na_2{}^{35}SO$ and an i.v injection of H-NA The levels ...endogenous NA in the atrial tissue from gan- ...glionectomized cats were significantly reduced (0.28±0.03 μg/g tissue) compared with controls (1.59±0.2 μg/g tissue)

The ^{3}H NA labelled subcellular particles from atrial tissue of control cats showed on continuous sucrose gradients a distribution concomitant with ^{35}S (Fig 1*a*). The peak was located approximately at 1.2 M sucrose. After ganglionectomy the distribution pattern of both ^{3}H and ^{35}S remained essentially unchanged (Fig. 1*b*) the areas under the

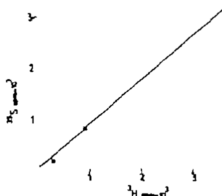


Fig 2 Scatter diagram of in vivo uptake of H-NA and incorporation of ^{35}S-sulphate into noradrenergic vesicle-containing gradient fractions from control (O) and ganglionectomized (●) cats. Correlation coefficient = 0.96, significant at $P<0.01$

identical particles The present experiments were performed in order to get further information about the localization of the particle bound SMPS The effects of surgical sympathectomy on adult animals were compared with the effects of neonatal treatment with 6-hydroxydopamine (6-OH DA) and with guanethidine

Whereas ganglionectomy decreased ^{3}H noradrenaline (NA) uptake and ^{35}S incorporation into noradrenergic vesicle fractions from cat hearts in a parallel way neonatal treatment with 6-OH DA and with guanethidine lowered the content of endogenous NA and reduced the uptake of ^{3}H NA—more so after 6-OH DA than after guanethidine—without a concomitant reduction of the incorporation of ^{35}S

METHODS

Experimental

Surgical sympathectomy in adult cat The right and left stellate ganglia were removed in two sessions within 2–3 weeks cats (5–3 kg) premedicated with atropine (15 μg/kg) were anesthesized with sodium pentobarbital (30 mg/kg i p) and intubated Artificial respiration was maintained with a Brown Melsungen ventilator The thorax was opened under sterile conditions between the second and the third ribs the stellate ganglia tied off and removed Penicillin was given for 6 days (15 000 I U./kg i m) Three weeks after the removal of the left stellate ganglion the cats were injected s c with 8 mCi/kg of $Na_2{}^{35}SO_4$ (carrier-free Kjeller Norway) and i v with 50 μCi/kg of dl-7 H NA (13 6 Ci/mmol New England Corp USA) 4 h and 1.5 h before sacrifice respectively Control cats received the same sequence of injections The heart was removed in pentobarbital anesthesia and the atria were dissected on ice Major portions from both atria were taken for isolation of noradrenergic vesicle fractions minor portions for determination of NA

Administration of 6-OH DA and of guanethidine to newborn rats

6-OH DA (H 88/3 AB Hässle) was dissolved immediately before use in ice-cold saline that had been saturated with nitrogen. The substance was injected s c in a dose of 100 mg/kg 8 h and 4 h after birth and in a dose of 250 mg/kg on the 8th and 15th day after birth (Provost et al 1974) Guanethidine (Ciba Göteborg) was dissolved in redistilled water immediately before use and the pH adjusted to 7 4 Doses of 50 mg/kg/day were administered s c for 15 days the first injection within 4 h after birth (essentially according to Johnson et al 1975) Control animals for both groups received parallel injection sequences of saline

The animals were sacrificed at the age of 10 weeks by cervical dislocation under light chloroform anesthesia. To one group of rats (chosen at random from the treated and control animals) Na_2 SO (carrier-free) was given s c in a dose of 8 mCi/kg and dl-7 ^{3}H-NA (6-10 Ci/ i v in a dose of 100 μCi/kg, 4 h and 1 h before sacrifice respectively Organs were taken for isolation of subcellular fractions containing noradrenergic vesicles F another group of treated and control rats, organs taken for determination of endogenous NA.

Isolation of subcellular fractions

Cat tissue Atrial tissue was homogenized in 10 vol of an isotonic solution of sucrose buffered to pH 7 4 a potassium phosphate buffer using a Potter Elvehjem homogenizer with a glass pestle. The homogenate centrifuged for 15 min at 8 000 g_{max} the resulting supernatant for 30 min at 125 000 g_{max} The final pellet resuspended in 0.25 M sucrose and layered on a continuous density gradient from 0.25 to 1 8 M sucrose Following centrifugation for 30 min at 175 000 g_{max} drops fractions were collected through the pierced bottom of the tube The radioactivity due to ^{3}H and ^{35}S determined in the fractions

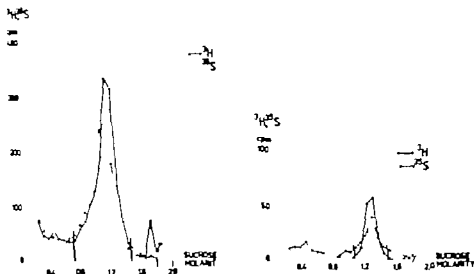
Rat tissue Immediately after sacrifice, heart, submaxillary glands, seminal ducts and adrenal glands were removed and chopped on ice. The organs were homogenized in 8 volumes of buffered isotonic sucrose (pH 7 4) using an Ultra Turrax homogenizer for [illegible] 45.5 V The homogenates were centrifuged for 15 min at 15 000 g_{max} the resulting supernatant for 45 min at [illegible] g_{max} The final pellet was resuspended in 500 μl of [illegible] M sucrose. This resuspension which contains [illegible] adrenergic vesicles contaminated mainly with [illegible] somes is henceforth referred to as $P_{[illegible]}$ Aliquots of [illegible] resuspension were taken for determination of [illegible] and S radioactivity protein and for extraction of [illegible] A large granule fractions containing chromaffin [illegible] was isolated from the adrenal glands essentially according to Banks (1965) and aliquots were taken for measurements as above

Assay

Endogenous NA was extracted from tissues by homogenization in 0 4 M perchloric acid separated by chromatography on Al_2O_3 and assayed by the trihydroxyindole method (Udenfriend 1962) The recovery of NA was 103 % In the adrenals both NA and adrenaline (A) were differentiated by oxidation at pH 6 5 and 3.5 respectively the overlap being corrected by means of internal standard The sum of NA and A is denoted as catecholamines (CA) Protein was determined according to Lowry et al (1951)

Measurement of radioactivity 10 ml Instagel® (Packard) was added to aliquots of subcellular particle suspensions and the radioactivity was measured by liquid scintillation spectrometry (Packard spectrometer [illegible] Using the external standard system double channel [illegible] was applied for distinction of H and ^{35}S radioactivity Corrections were made for quenching and overlap between channel

Extraction of 35-labelled lipid Aliquots of the cellular resuspensions were placed on Munktell's extraction thimbles and dried in vacuo over phosphorus pentoxide at 4°C The lipid [illegible] ucted [illegible]



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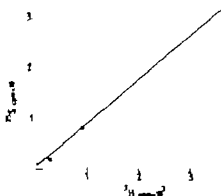


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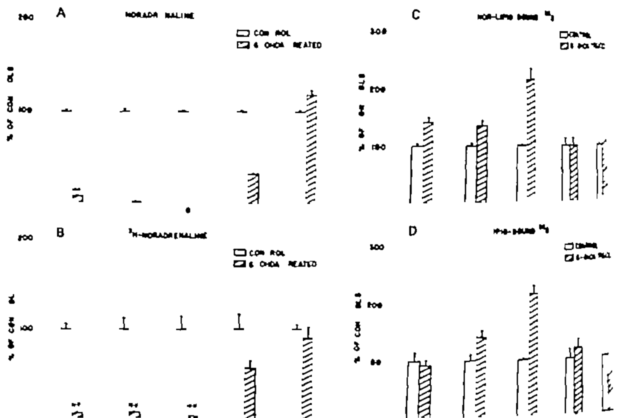


Fig 3a–d Effect of neonatal treatment of rats with 6-OH DA (100 mg/kg 8 h and 24 h, 50 mg/kg on the ... after birth) on (*a*) tissue contents of endogenous NA (*b*) ³H NA uptake into the $P_{155\,000}$ fractions, (*c*) ³⁵S-sulphate incorporation into non-lipid compounds (SMPSs) of the $P_{155\,000}$ fractions and (*d*) ³⁵S-sulphate incorporation into lipids of the $P_{155\,000}$ fractions from the heart (*H*) spleen (*S*) submaxillary glands (*G*) seminal ducts (*V*) and adrenal glands (...) 10-week-old rats. Values are expressed as percentages of the respective controls. Means ± S.E. of 6 animals ... $P<0.01$ $P<0.001$

peaks decreased to 35% and 41% of controls for ³H and ³⁵S respectively Both in control and in ganglionectomized cats the incorporation of ³⁵S and the ³H NA uptake into fractions containing nor adrenergic vesicles were highly correlated (Fig 2)

Effects of neonatal treatment with 6-OH DA and with guanethidine (rats)

a) *Survival and growth* The administration of 6-OH DA or guanethidine to newborn rats resulted in a high mortality rate 64% and 70% respectively compared with corresponding saline-treated groups (31% and 54% respectively) In all groups death occurred mainly during the first two weeks The high death rates were limited to certain litters where mothers refused to accept the pups after treatment

The growth rate of animals treated with the neurotoxic compounds was inhibited (by 30% and 40% in the 6-OH DA and guanethidine-treated groups respectively) during the first 4 weeks. At the age of 10 weeks however the body w... of treated animals was reduced by only 15% ... 6-OH DA treated rats exhibited a permanent p... and enophthalmos until sacrifice guanethi... treated rats showed only a transient ptosis for ... weeks at the age of 4 weeks

b) *Levels of endogenous NA* Both control ... treated rats were sacrificed at the age of 10 wee... following a s c. injection of $Na_2{}^{35}SO_4$ and ... injection of ³H NA In 6-OH DA treated rats (F... 3*a*) the levels of endogenous NA were stro... reduced in the heart spleen and submax... glands (to 8%) but less so in the seminal d... (to 25%) On the other hand the adrenals cont... more CA than controls. In guanethidine-tr... rats (Fig 4*a*) the NA levels in the he... and submaxillary glands were reduc... in the seminal ducts only to 45... decrease in the CA ... he

c) *In vivo upta...*

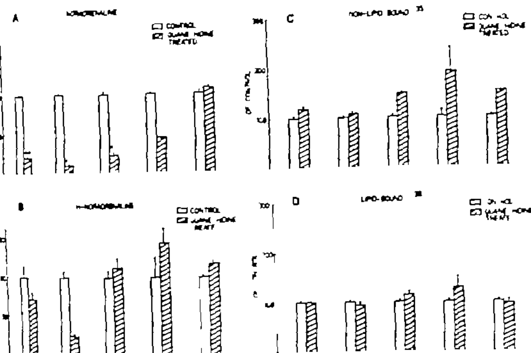


Fig. 4*a–d*. Effects of neonatal treatment of rats with guanethidine (30 mg/kg) daily for 15 days, starting within 24 h after birth on (*a*) tissue contents of endogenous NA, (*b*) ³H-NA uptake into the $P_{135\,000}$ fractions, (*c*) ³⁵S-sulphate incorporation into nonlipid compounds (SMPSs) of the $P_{135\,000}$ fractions and (*d*) ³⁵S-sulphate incorporation into lipids of the $P_{135\,000}$ fractions from the heart (*H*), spleen (*S*), submaxillary glands (*G*), seminal ducts (*V*) and adrenal glands (*A*) of 10-week old rats. Values are expressed as percentages of the respective controls. Means ± S.E. from 6 animals are given. *P<0.01, **P<0.001

treated rats (Fig. 3*b*) in vivo uptake of ³H-NA into the $P_{135\,000}$ fractions from the heart, spleen and submaxillary glands was strongly reduced (to 3–...). However, in fractions isolated from the seminal ducts the uptake was decreased only to 60%. The uptake into chromaffin vesicles from the adrenals was unaffected. In guanethidine-treated rats (Fig. 4*b*) in vivo uptake of ³H-NA into the $P_{135\,000}$ fractions was significantly reduced only in the spleen (to 20%) and somewhat lowered in the heart; it remained unchanged or even increased in the submaxillary glands, seminal ducts and the adrenals.

In vivo incorporation of ³⁵S. Incorporation of ³⁵S into non-lipid compounds of the $P_{135\,000}$ fractions was generally higher in males than in females (results not shown). Both after treatment with 6-OH DA (Fig. 3*c*) and after guanethidine (Fig. 4*c*) the incorporation of ³⁵S-sulphate into non-lipid compounds of the $P_{135\,000}$ fractions from all examined organs was enhanced, most conspicuously in the submaxillary glands. An increased incorporation of ³⁵S was also observed in the chromaffin vesicles of the adrenals.

In both 6-OH DA (Fig. 3*d*) and guanethidine (Fig. 4*d*) treated rats in vivo incorporation of ³⁵S-sulphate into lipids of the $P_{135\,000}$ fractions from all examined organs was essentially unchanged except for an increase in the submaxillary glands of the 6-OH-DA treated rats.

The ratios of non-lipid to lipid ³⁵S in saline-treated controls varied between organs and between sexes (Table 1); the highest ratio was found in the heart, the lowest in the submaxillary glands. The ratios tended to be higher in guanethidine treated rats, most so in the adrenal glands.

A comparison of the effects of different sympathectomies is presented in Table 2. All 3 kinds of denervation substantially depleted the stores of NA in sympathetic nerve terminals (most

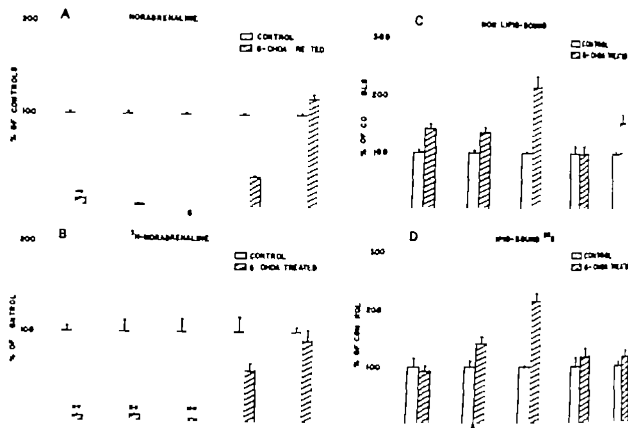


Fig 3a–d Effect of neonatal treatment of rats with 6-OH DA (100 mg/kg 8 h and 4 h ..50 mg/kg on the 8th and 15th after birth) on (*a*) tissue contents of endogenous NA, (*b*) H NA uptake into the P_{1} fractions, (*c*) ^{35}S-sulphate incorporation into non-lipid compounds (SMPSs) of the P_{1} fractions and (*d*) ^{35}S-sulphate incorporation into lipids of the P_{1} fractions from the heart (*H*) spleen (*S*) submaxillary glands (*G*) seminal ducts (*V*) and adrenal glands (*A*) of 10-week-old rats. Values are expressed as percentages of the respective controls. Means ± S.E. of 6 animals are given. $P<0.01$ $P<0.001$

peaks decreased to 35% and 41% of controls for ^{3}H and ^{35}S respectively Both in control and in ganglionectomized cats the incorporation of ^{35}S and the ^{3}H NA uptake into fractions containing nor adrenergic vesicles were highly correlated (Fig 2)

Effects of neonatal treatment with 6-OH DA and with guanethidine (rats)

a) *Survival and growth* The administration of 6-OH DA or guanethidine to newborn rats resulted in a high mortality rate 64% and 70% respectively compared with corresponding saline-treated groups (31% and 54% respectively) In all groups death occurred mainly during the first two weeks The high death rates were limited to certain litters where mothers refused to accept the pups after treatment

The growth rate of animals treated with the neurotoxic compounds was inhibited (by 30% and 40% in the 6-OH DA and guanethidine treated groups respectively) during the first 4 weeks At the age of 10 weeks however the body weight of treated animals was reduced by only 15% ... 6-OH DA treated rats exhibited a permanent ptosis and enophthalmos until sacrifice guanethidine treated rats showed only a transient ptosis for ... weeks at the age of 4 weeks

b) *Levels of endogenous NA* Both control and treated rats were sacrificed at the age of 10 weeks following a s c injection of $Na_2{}^{35}SO$ and an i... injection of H NA In 6-OH DA treated rats (Fig 3*a*) the levels of endogenous NA were strongly reduced in the heart spleen and submaxillary glands (to 8%) but less so in the seminal ducts (to ..5%) On the other hand the adrenals contained more CA than controls In guanethidine-treated rats (Fig 4*a*) the NA levels in the heart spleen and submaxillary glands were reduced to 10-15% in the seminal ducts only to 45% There was a decrease in the CA levels of the adrenal

c) *In vivo uptake of exogenous H*

...ring & Schlefthaler (1964). After stellate gan-...nectomy these authors found a parallel de...se in endogenous NA and in the uptake of H-..., while we did not. The discrepancy might be ...to an incipient regeneration of some nerve ...nals in our experiments since we sacrificed ...animals 5–6 weeks after denervation, whereas ...rting & Schlefthaler waited only 10 days before ...rifice.

The density gradient distribution of the ^{3}H NA ...among subcellular particles from homogenized ...ervated and denervated atria was identical, in ...ordance with the assumption that in both cases ...^{3}H-NA is localized to noradrenergic vesicles. ...bout 70% of the total ^{35}S in noradrenergic vesicle ...ctions from cat atria has been found to be present ...non-lipid compounds (Blaschke et al. 1976). ...cordingly the parallel gradient distribution of ...H NA and ^{35}S in the present experiments is in ...reement with our previous proposals that nor...renergic vesicles contain SMPSs (Åborg et al. ...72, Blaschke et al. 1976). The concomitant ...duction of the accumulation of ^{3}H NA and ^{35}S ...the noradrenergic vesicle fractions after sym...thectomy further supports this idea. However ...x gradient data have to be interpreted with cau...on. There might be other ^{35}S-accumulating sub...cellular particles of similar gradient characteristics ...s noradrenergic vesicles. SMPSs have been found ...microsomes from neurons (for ref. see Margolis ...& Margolis 1977) as well as other cells (Robinson & ...rera 1962, Baumgartner et al. 1974). Although it ...is therefore not possible to contend that the reduced ...corporation of ^{35}S reflects only changes in SMPS ...ontents of noradrenergic vesicles, the high correla...tion between the accumulation of ^{3}H-NA and ^{35}S ...before and after denervation provides suggestive ...dence for this possibility.

Following administration of 6-OH DA and of ...guanethidine to newborn rats the contents of endo...genous NA in the various organs studied were re...duced to different extents. The depletion of NA was ...highest in the spleen and salivary glands, lower in ...the heart and seminal ducts. It was somewhat less ...marked after guanethidine than after 6-OH-DA. ...The CA contents in the adrenals were unchanged ...or slightly increased. Our results in 6-OH DA ...treated rats are in good agreement with those of ...previous investigators (Kostrzewa & Jacobowitz ...1974, Singh & De Champlain 1974, Provost et al. ...1974, Jacobowitz 1975). After guanethidine, levels of NA were slightly higher than those reported by Johnson et al. (1975).

In the 6-OH DA-treated rats the in vivo uptake of ^{3}H NA into the $P_{1\,000}$ fractions of the heart, spleen and salivary glands was lowered approximately parallel with the endogenous NA but less so in the seminal ducts (by 43% vs. 77%). After treatment with guanethidine the uptake was affected much less, except for the uptake into the spleen. It equalled or even exceeded control values (in the salivary glands and seminal ducts, respectively). Accordingly there were considerable discrepancies between changes in the storage of endogenous NA and changes in the uptake of exogenous NA in the various organs, especially after guanethidine. It is not easy to explain this finding because our knowledge of the biochemical events behind the neurotoxic actions of the drugs is very imperfect. Since ^{3}H NA uptake into particulate fractions was measured, extraneuronal uptake can be ruled out (for ref. see Euler 1972). A possible reason for the discrepancies might be differences in the extent of regeneration that follows drug treatment.

Studies both of ganglia and of innervation areas over long periods revealed apparently intact nerve cells after neonatal treatment with 6-OH DA and with guanethidine; the neurons are able to proliferate, partially innervating the corresponding tissue (Jaim-Etcheverry & Zieher 1971, Eränkö & Eränkö 1972, Singh & de Champlain 1974, Jacobowitz 1975, Johnson et al. 1975, 1976). However, in comparison to mature terminals the new fibres display a weaker fluorescence (Jacobowitz 1975), resembling the postnatal ontogenesis of sympathetic neurons (Schaebler & Heene 1968). This has been tentatively explained as due to a more ramified and extended network of fibres to compensate for the destroyed terminals and/or an increased turnover rate of NA in these fibres (Olsson & Jacobowitz, respectively, in de Champlain 1975). The regeneration process resembles ontogenesis in another aspect, too. Just as tissues develop the ability to take up exogenous NA at all times prior to the synthesis and storage of NA (Iversen et al. 1967), neurons regenerating after destruction of their terminals contain less NA than mature fibres but the efficiency of the uptake equals that of the mature state (Jonsson & Sachs 1970, Jonsson 1974). The uptake capacity might reflect the actual number of nerves (Sachs et al. 1970).

Analogously the high accumulation of exogen-

Table 1 *Ratios of non lipid bound ³⁵S to lipid bound ³⁵S in the $P_{135\,000}$ fractions (see Methods) from organ homogenates of control rats and rats treated neonatally with 6-OH DA (100 mg/kg 8 h and 24 h 250 mg/kg on the 8th and 15th day after birth) or guanethidine (50 mg/kg daily for 15 days starting within 24 h after birth)*

The rats were sacrificed at the age of 10 weeks. Means ± S E of 3–4 animals are given

	Control	6-OH DA	Guanethidine
Female			
Heart	4 0±0 4	2.4±0 3	5.5±0 3
Spleen	3 4±0.0	3.5±0 2	3 8±0.1
Submaxill gl.	0.3±0 0	0 3±0 0	0 4±0 0
Adrenals	3 2±0.2	3 6±0 4	5 1±0 3 *
Male			
Heart	6.2±0 6	9 9±0.3	7 7±0 3
Spleen	5 5±0.3	6.1±0 1	6.6±0.2
Submaxill gl	0 4±0 0	0.4±0 0+	0.5±0 0
Seminal ducts	1 1±0 1	1 0±0 0	1 1±0 1
Adrenals	6 7±0 3	8 9±0 4	10 8±0.8

$P<0.01$ * $P<0.005$

markedly in tissues innervated by long postganglionic fibres such as the heart less in tissues innervated by short fibres such as the seminal ducts) The levels of CA and the uptake of exogenous NA into adrenal glands were both unaffected or slightly increased

On the other hand the effect of the 3 denervation procedures on the uptake of exogenous ³H into the $P_{135\,000}$ fractions from sympathetically innervated tissues was differentiated: while treatment with 6-OH DA substantially reduced the uptake in most organs examined) guanethidine largely failed to affect the uptake except for the splenic sympathetic system Five weeks after ganglionectomy the uptake into vesicles of the cat heart varied from low to slightly decreased

Both kinds of neonatal sympathectomy resulted in normal or slightly increased incorporation of ³⁵S-sulphate into nonlipid compounds of the $P_{135\,000}$ fractions from sympathetically innervated tissues as well as of the chromaffin vesicles from the adrenal glands Following ganglionectomy the reduction of the total ³⁵S-incorporation appeared highly correlated to the reduction of the ³H-NA uptake

DISCUSSION

Five to six weeks after bilateral stellate ganglionectomy the content of endogenous NA in cats atria was lowered to ~20% of the control level, while the ability of the noradrenergic vesicle-containing fractions to accumulate ³H NA and to incorporate ³⁵S was reduced to 35% and 31% of the control values respectively There is a minor discrepancy between our data on H NA uptake and those

Table 2 *Effects of different sympathectomies on the storage and uptake of NA and on the incorporation of ³⁵S sulphate into SMPSs in noradrenergic vesicle-containing fractions from various organs*

Bilateral stellate ganglionectomy was performed on adult rats 5–6 weeks before sacrifice. 6-OH DA was administered to rats 8 h and 24 h (100 mg/kg) and on the 8th and 15th day (250 mg/kg) after birth. Guanethidine was administered to rats daily for 15 days starting within 24 h after birth (50 mg/kg) The rats were sacrificed at the age of 10 weeks
+ = increase – = decrease 0 = no effect

	Ganglion-ectomy	6-OH DA	Guanethidine
Endogenous NA			
In peripheral tissues	–	–	–
In adrenals	0	0	0
H NA uptake			
In peripheral tissues*	–	–	Spleen – other 0 or +
In adrenals		0	0
³⁵S incorporation into SMPSs of peripheral tissues* and adrenals	–	+ or 0	+

Heart, spleen submaxillary glands and seminal ducts
* Gradient fractions from cat atria, $P_{135\,000}$ fractions from rat heart, spleen submaxillary glands and seminal d (see methods)
Chromaffin vesicles from adrenal glands

-ting & Schiefthaler (1964). After stellate gan-ectomy these authors found a parallel de-se in endogenous NA and in the uptake of H while we did not. The discrepancy might be to an incipient regeneration of some nerve ...als in our experiments, since we sacrificed animals 5–6 weeks after denervation, whereas ...ting & Schiefthaler waited only 10 days before ...fice.

The density gradient distribution of the ^{3}H-NA among subcellular particles from homogenized ...ervated and denervated atria was identical, in ...ordance with the assumption that in both cases ^{3}H-NA is localized to noradrenergic vesicles. ...out 70% of the total ^{35}S in noradrenergic vesicle ...ctions from cat atria has been found to be present ...non-lipid compounds (Blaschke et al. 1976). ...cordingly the parallel gradient distribution of ...NA and ^{35}S in the present experiments is in ...reement with our previous proposals that nor-...renergic vesicles contain SMPSs (Åborg et al. ...77, Blaschke et al. 1976). The concomitant ...duction of the accumulation of ^{3}H-NA and ^{35}S ...the noradrenergic vesicle fractions after sym-...thectomy further supports this idea. However ...r gradient data have to be interpreted with cau-...n. There might be other ^{35}S-accumulating sub-...llular particles of similar gradient characteristics ...s noradrenergic vesicles. SMPSs have been found ...microsomes from neurons (for ref. see Margolis ...Margolis 1977) as well as other cells (Robinson & ...reen 1962, Baumgartner et al. 1974). Although it ...therefore not possible to contend that the reduced ...corporation of ^{35}S reflects only changes in SMPS ...ontents of noradrenergic vesicles, the high correla-...on between the accumulation of ^{3}H NA and ^{35}S ...before and after denervation provides suggestive ...vidence for this possibility.

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Studies both of ganglia and of innervation areas over long periods revealed apparently intact nerve cells after neonatal treatment with 6-OH DA and with guanethidine: the neurons are able to pro-liferate partially innervating the corresponding tissue (Jaim-Etcheverry & Zieher 1971, Eränkö & Eränkö 1972, Singh & de Champlain 1974, Jacobo-witz 1975, Johnson et al. 1975, 1976). However in comparison to mature terminals the new fibres display a weaker fluorescence (Jacobowitz 1975) resembling the postnatal ontogenesis of sympathe-tic neurons (Schiebler & Heene 1968). This has been tentatively explained as due to a more rami-fied and extended network of fibres to compensate for the destroyed terminals and/or an increased turnover rate of NA in these fibres (Olsson & Jaco-bowitz, respectively, in de Champlain 1975). The regeneration process resembles ontogenesis in another aspect, too. Just as tissues develop the ability to take up exogenous NA at all times prior to the synthesis and storage of NA (Iversen et al. 1967), neurons regenerating after destruction of their terminals contain less NA than mature fibres but the efficiency of the uptake equals that of the mature state (Jonsson & Sachs 1970, Jonsson 1974). The uptake capacity might reflect the actual num-ber of nerves (Sachs et al. 1970).

Analogously the high accumulation of exogen-

ous NA in the $P_{25\,000}$ fractions 8 weeks after the end of guanethidine administration might reflect the density of the new terminal plexus Since the young fibres may not yet be able to synthesize and store NA to the full extent the tissue contents of NA are low

The non-lipid ^{35}S-labelled compounds in the $P_{135\,000}$ fractions correspond presumably to SMPSs and possibly sulphated glycoproteins ^{35}S-sulphate has been shown to be incorporated into these macromolecules in biogenic-amine storing organelles from the adrenals (Fillion et al 1971 Geissler et al 1977) from sympathetic nerves (Åborg et al 1972 Blaschke et al 1976) and from the brain (Robinson & Green 1962 Pycock et al 1975 incl references) In both the 6-OH DA and the guanethidine-treated rats the rate of ^{35}S-incorporation into SMPSs of the $P_{135\,000}$ fractions was in most organs considerably less depressed than the ^{3}H NA uptake—in contrast to the parallel changes of the two processes in atrial tissue after stellate ganglionectomy The reason for these discrepant effects of surgical and pharmacological denervation" can be only speculated upon

A plausible explanation seems to be a high rate of ^{35}S-sulphate incorporation into SMPSs of regenerating nerve terminals Outgrowing neurons show a high rate of ^{35}S incorporation into SMPSs (Guha et al 1962 Margolis et al 1975) The most likely site of the SMPS synthesis is microsomal structures from which the newly synthesized SMPSs are rapidly transferred to storage organelles as e g leukocyte granules (Olsson 1969) or chromaffin vesicles (Geissler et al 1977) By analogy a similar transfer might be assumed to occur in regenerating neurons To what extent the ^{35}S in the present experiments is localized to noradrenergic vesicles will remain unclear until such vesicles can be obtained with satisfactory purity However the assumption that the ^{35}S corresponds to newly formed SMPSs in regenerating vesicles agrees well with the low contents of endogenous NA and the relatively high uptake capacity for exogenous NA in the regenerating dense network of nerve terminals as discussed above

In summary The parallel effects of sympathectomy of the cat s heart on the accumulation of ^{3}H NA and ^{35}S in particulate gradient fractions support the assumption that SMPSs are localized to noradrenergic vesicles. The discrepant effects of neonatally administered 6-OH DA and guanethidine on the accumulation of ^{3}H NA and the incorporation of ^{35}S in particulate fractions from various organs of the rat are tentatively explained as due to regeneration phenomena. The functional significance of the SMPSs in noradrenergic vesicles for the storage and release of NA will be discussed in future papers from this laboratory

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'diovascular reactivity' to graded splanchnic nerve nulation in spontaneously hypertensive and rmotensive control rats

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NORESSON E., FOLKOW B. & HALLBÄCK-NORDLANDER, M.. Cardiovascular reactivity' to graded splanchnic nerve stimulation in spontaneously hypertensive and normotensive control rats. Acta Physiol Scand 1979 106: 169–176. Received 17 Nov 1978. ISSN 0001-6772. Department of Physiology University of Goteborg, Sweden.

Cardiovascular reactivity to graded splanchnic nerve stimulations was compared in adult spontaneously hypertensive rats (SHR) and normotensive controls (NCR), during abolished adrenal medullary secretion and neurogenic cardiac control and depressed reflex vascular adjustments. Arterial pressure, heart rate and cardiac output were measured, and total peripheral resistance (TPR) and stroke volume (SV) computed before, during and after nerve stimulation. The neurogenic resistance increases in the major gastrointestinal-renal hepatic circuits expressed themselves as TPR elevations, which were much accentuated in SHR. This reflects an increased h_r of SHR resistance vessels rather than any altered effector sensitivity since the responses were particularly accentuated at high discharge rates when noradrenaline junction concentrations approach maximal levels. The splanchnic capacitance responses expressed themselves as SV increases, being the most relevant aspect of capacitance control. SV increased less in SHR, mainly reflecting the reduced diastolic compliance of the hypertrophied SHR left ventricle and the consequent rightward shift of its Frank-Starling curve. The results indicate that an elevated resistance may well be maintained by normal sympathetic discharge in established SHR hypertension. There seems, however to be an increasing need for accentuated discharge to the capacitance side to maintain proper cardiac filling of the hypertrophied left ventricle.

Key words. Cardiovascular reactivity left ventricular hypertrophy primary hypertension, splanchnic nerve stimulation, spontaneously hypertensive rats, stroke volume, structural cardiovascular resetting, total peripheral resistance

posure to frequent episodes of environmental ess situations can in experimental animals dually lead to elevations also of resting mean erial pressure (MAP) (e g Folkow & Rubinstein 56, Henry Meehan & Stephens 1967) Thus sug-sts the involvement also of chronic cardiovascu- adjustments of non-neurogenic nature in such pes of neurogenic hypertension Concerning the ost commonly used animal model of man's es-ntial hypertension the Okamoto-Aoki type of ontaneously hypertensive rat (SHR) many udies indicate that they exhibit an accentuated, eurohormonally mediated cardiovascular reactivi- to environmental alerting stimuli (cf. Yamori et l. 1969 Okamoto 1972, Hallback & Folkow 1974) his implies amplified though transient MAP eleva-ons in connection with ordinary daily life stimuli) turn tending to elevate average MAP during early developing phases of this variant of primary hypertension (cf Hallbäck 1975). There is by now strong evidence that any type of more prolonged *MAP* elevation, thus also intermittent ones if only repeated often enough can trigger a gradual structural adaptation of the pressure-exposed left heart, systemic arteries and arterioles in the direc tion of wall hypertrophy in relation to lumen which, in turn, becomes responsible of a new hemodynamic equilibrium at a higher MAP level (Folkow Grimby & Thulesius 1958, Folkow et al. 1973 1974). A similar chain of events seems to be of relevance also for the initiating phases of human essential hypertension at least in some of its more common variants (cf. Folkow 1975 Julius & Esler 1976)

Concerning established hypertension in man it has, however been widely assumed that the

chronically elevated resistance and MAP levels should also during this phase have a functional background This has resulted in an intense search for increased concentrations of bloodborne pressor agents and/or signs of a continuously enhanced sympathetic activity though largely in vain Concerning SHR on the other hand it was recently postulated that a tonic increase of sympathetic activity should indeed be responsible of the raised resistance and MAP levels also in the established phase (Iriuchijima 1973) Thus section of the splanchnic nerves led to equally low MAP levels in SHR and normotensive controls (NCR) and moreover a definitely higher frequency of splanchnic stimulation was needed in SHR to restore MAP to initial levels.

However MAP depends not only on total peripheral resistance (TPR) but also on cardiac output (CO) and the latter parameter must of course also be taken into account For example left ventricular hypertrophy means a reduced diastolic compliance which calls for an elevated enddiastolic pressure and/or an increased inotropic state in SHR to maintain the same stroke volume (SV) as in NCR (Hallbäck Isaksson & Noresson 1975 Noresson et al 1979) Further it was recently shown in intact adult SHR and NCR that left enddiastolic pressure is indeed about doubled in SHR (Noresson Ricksten & Thorén 1979) Evidently in established hypertension the entire low-pressure compartment with its important capacitance function for the left ventricle becomes secondarily affected because of the reduced left ventricular diastolic compliance For such reasons major interferences with the voluminous splanchnic capacitance section and hence with SV and CO must have been involved in Iriuchijima s experiments suggesting that the MAP alterations had a more complex hemodynamic background than merely reflecting parallel changes of TPR. Furthermore a closer analysis of the equally low MAP levels reached in SHR and NCR after complete sympathetic destruction by pithing reveals that TPR remains about 35% higher in SHR while CO is about 35% lower in SHR than in NCR (Albrecht et al 1975)

The present experiments were designed to explore whether and to what an extent SHR and NCR differ with respect to both neurogenic resistance and capacitance responses and whether such differences could explain Iriuchijima s findings Towards such a background it might also be possible to state whether there really is any evidence [illegible] accentuated sympathetic vasoconstrictor fibre [illegible] tivity to account for the TPR and MAP elevation [illegible] established SHR hypertension Part of the present results have been briefly reported earlier (Folkow Hallbäck & Noresson 1977)

METHODS

25 5–6 months old male SHR (357±10 g) and 25 male NCR (369±7 g) were used. During light ether [illegible] PE 50 catheter was inserted into the tail artery [illegible] the animal was allowed to wake up. Awake mean ar[illegible] pressure (MAP) and heart rate were then recorded [illegible] Statham 23DC pressure transducer writing on [illegible] polygraph

Preparation The rats were given heparin ([illegible] b w) and anesthetized with nembutal (40–50 mg/[illegible] 1/4 of the nembutal dose was given additional[illegible] needed Nembutal markedly depresses reflex [illegible] vascular adjustments in rats (Noresson, Jones [illegible] bäck 1977) which for these experiments was [illegible] advantage The left femoral artery and vein were [illegible] lated with PE 50 catheters for cardiac output (CO) [illegible] urements. Another PE 50 tube was inserted in the jugular vein to measure central venous pressure [illegible] however the baseline was so unstable that [illegible] during splanchnic nerve stimulations were diffic[illegible] quantify After these cannulations the abdomen [illegible] opened via a midline and a subcostal cross [illegible] splanchnic nerves were bilaterally dissected fr[illegible] proximal to the coeliac ganglion and the adrenal [illegible] were cautiously freed from the nerves and [illegible] fine ligatures. The splanchnic nerves were cut bi[illegible] and the distal ends placed in silver ring electrodes [illegible] were carefully isolated from surrounding tissues.

Propranolol was then administered every 30 min, [illegible] ly 3 mg/kg b w and thereafter 0.5–1 mg/kg b. [illegible] was administered on an equal time schedule, [illegible] mg/kg b w and subsequently 0.–0.3 mg/kg b w [illegible] this pharmacologic blockade of the efferent cardiac [illegible] vation, heart rate remained entirely unchanged [illegible] splanchnic stimulations During the whole prep[illegible] MAP and heart rate were continuously recorded [illegible] intermittently measured Throughout the experiment [illegible] animals were breathing spontaneously In some [illegible] hematocrit was measured both before and after the [illegible] experiment but changes beyond 5% were not observ[illegible]

Cardiac output measurement The Cardiogreen [illegible] dilution technique was used to measure CO By mean[illegible] step dispenser syringe 0.043 ml of Cardiogreen sol[illegible] (2.3 mg/ml) was injected into the femoral vein. Art[illegible] blood, for each measurement at most 0 5 ml, was [illegible] drawn at a constant speed of 1 2 ml/min passing thr[illegible] a pecially designed low volume (0.05 ml) densitom[illegible] curvette after which the blood was immediately [illegible] fused Calibration of the densitometer was performed [illegible] known concentrations of dye diluted in filtered [illegible] plasma. The system was linear over the recording [illegible] used The dye-dilution curves were mathema[illegible]

1 Gives the mean arterial pressure in intact awake rats together with values for mean arterial ...ee cardiac output (CO), stroke volume (SV) and total peripheral resistance (TRP) (mean±S.E.), ...y Nembutal® anesthesia before the abdomen is open

... right is given left ventricular weight in per cent of body weight. Levels of significant differences between groups ...ut below (*$P<0.05$ ** $P<0.001$) as well as the ratio between SHR and NCR

	Awake	During nembutal anaesthesia				
	Mean arterial blood pressure (mmHg)	Mean arterial blood pressure (mmHg)	CO (ml/min × 100 g b.w.)	SV (µl/100 g b.w.)	TPR (mmHg/ml/min 100 g b.w.)	Left ventricular weight in per cent of body weight
[illegible]	169±4	186±4	20.9±0.9	56.9±2.1	9.0±0.5	0.27±0.01
[illegible]	121±2	134±8	4.1±1.2	60.0±3.8	5.6±0.3	0.19±0.01
	**	***		n.s.	***	
NCR	1.39	1.39	0.87	0.95	1.61	1.42

...ed using a digitizer unit connected to Hewlett ...rd calculator (cf. Albrecht et al. 1975)

...rimental protocol During the experiment, which ... 130 min, both splanchnic nerves were supramaximal... ...mulated (8 ms, 15 V) with increasing frequencies (2, 4, 8, 16 and 32 Hz) using a square wave stimulator ... only one of the nerves was stimulated the MAP ...nse was about 3/4 of that obtained when both ...s were stimulated indicating a marked overlap ...een the right and left splanchnic nerves (Irikuchijima ...). At each frequency the nerves were stimulated for 2 ... and stimulations were repeated every third to fourth ... Between and during each stimulation two CO measure... ...nts were performed. The mean of these measure... ...ts was used and the stimulation effect was compared ...the control level during steady state after each stimula... ...n. In this way the neurogenic increases in MAP and CO ...well as in SV and TPR per 100 g b.w. were obtained. ... the course of the stimulation period the CO elevation ...nded to fall slightly and therefore the neurogenic in... ...ses in SV, TRP and MAP were throughout related to ...d resting control values just after the stimulations.

...V and TPR were calculated from the CO, MAP and ...rt rate measurements and relative changes as well as ...these values were determined in each animal. Mean ...es and levels of significant difference between groups ... calculated according to the group comparison Stu... ...t' -test. Values are presented as mean ± S.E.

...SULTS

...the awake state mean arterial pressure (MAP) ... = 169±4 mmHg in SHR and 122±2 mmHg in ...R. During Nembutal anesthesia, but before au... ...tonomic heart blockade and abdominal surgery the ...MAP levels were slightly higher 186±4 mmHg in ...HR and 134±8 mmHg in NCR. In this situation ...tal peripheral resistance (TPR) was much higher in SHR than in NCR while cardiac output (CO) was some 15% lower in SHR than in NCR ($P<0.05$). Stroke volume (SV) was about equal in both since 'basal' heart rate was modestly lower in SHR in this situation (Table 1).

After the abdominal opening, nerve preparation and adrenal ligation which all together took about 60 min and included the pharmacological blockade of the efferent cardiac innervation MAP had fallen to 110±4 mmHg in SHR and to 94±5 mmHg in NCR. In SHR a considerable part of this pressure drop, 32 mmHg, occurred when the intraabdominal pressure was *reduced by opening the abdominal* cavity which only little affected MAP in NCR (8 mmHg). This procedure alone thus reduced the MAP levels to 154±4 mmHg *in SHR* and to 126±4 mmHg in NCR, while CO and SV fell by about 25% in both. The additional drop in MAP 45 mmHg in SHR and 32 mmHg in NCR, occurred in connection with the cardiac blockade, adrenalectomy and splanchnic nerve section.

Even though reflex cardiovascular adjustments are much depressed in rats during Nembutal® anesthesia, it is obvious that these marked MAP reductions must initiate some reflex changes in both *SHR* and *NCR*. For example, *TPR* increased to 15.2±0.8 PRU_{100} in SHR and to 10.2±0.6 PRU_{100} in NCR, while CO fell drastically in both to 9.3±0.8 ml/100 g b.w. in NCR and to 7.2±0.4 *ml/100 g b.w.* in SHR. Thus, compared with the preoperative situation (Table 1), SV fell from 60 to 25 µl in NCR and from 57 to 22.5 µl in SHR, despite reflex compensations in other areas. This reflects a consider

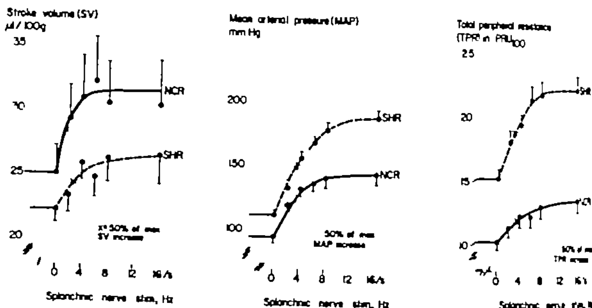


Fig 1 Left panel Increase of stroke volume (SV µl/100 g b w) in response to bilateral supramaximal splanchnic stimulation at increasing rates. Middle panel Increase of mean arterial pressure (MAP) in response to splanchnic stimulation at increasing rates Right panel Increase of total peripheral resistance (TPR) to increasing rates of spl nerve stimulation Each point represent the mean of values obtained in 17 rats and vertical bars gives the S E The lines indicate 50% of maximal increase Note the differences in frequency-response curve steepness between S NCR at low physiological' rates of vasoconstrictor fibre discharge Heart rate remained unchanged during spl nerve stimulation due to pharmacologic cardiac blockade

able reduction of venous return due to splanchnic pooling, in association with a reduced ventricular inotropism after cardiac sympathetic blockade to which comes unspecific interferences with myocardial function as a result of the operative procedures per se

The mean changes in SV MAP and TPR caused by splanchnic vasoconstrictor fibre stimulation at increasing frequencies are shown in Fig 1 for 17 pairs of SHR and NCR As seen from the left panel SV was initially about 10% higher in NCR than in SHR and it also increased more steeply in NCR at low rates of splanchnic nerve stimulation Thus half of the maximal SV increase produced by splanchnic nerve stimulation which in NCR amounted to 26% was reached already at 1 4 Hz while the corresponding figures for SHR were 19% and 2 8 Hz respectively Evidently the reduced diastolic compliance of the hypertrophied SHR left ventricle calls for an accentuated capacitance response to achieve a given diastolic prestretch and SV increase Here the consequent afterload increases must however also be considered but the finally reached MAP values during splanchnic stimulation exceeded the normal MAP or afterloads by only some 10–15% (187 versus 169 mmHg in SHR and 146 versus 122 mmHg in NCR) where the percentage afterload rise is, if any more marked in NCR than in SHR.

The right panel of Fig 1 shows th neurogenic resistance increases were more p ful in SHR than in NCR both in absolute and tive terms TPR increased maximally 3.% splanchnic nerve stimulation in NCR from a level of 10 2 PRU, while the correspondi values were a 47% increase from an initial l 15 2 PRU. Thus even in relative terms T creased about 50% more in SHR than in NC constrictor fibre stimulation despite a more increase in vascular transmural pressure. F the frequency-response curve for the SH sistance vessels was clearly steeper than the one and 50% of the maximal resistance was reached at a lower frequency in SHR t NCR

The neurogenic MAP increase shown in the dle panel of Fig. 1 is the product of the CO and increases The absolute MAP rise upon m splanchnic nerve stimulation was for SH mmHg (70%) and for NCR 49 mmHg (about To reach the control MAP present befo abdominal operation NCR had to be stim with 4 Hz but SHR with as much as 8 Hz. however here most im rtant to re

g of the abdominal cavity per se produced a reduction of 30 mmHg in SHR but only of 8 in NCR. Thus, the mentioned neurogenic or influence had then to compensate also for structural interference with cardiovascular ...

therefore much more fair to explore which ... stimulation frequencies that were ... to restitute the MAP levels present in SHR ... CR just after the abdominal opening, which 154 and 126 mmHg, respectively and where further pressure reduction is more directly to the eliminated splanchnic nerve influence. ...a comparison reveals that precisely the same of splanchnic nerve stimulation about 3.5 Hz ... then be needed in SHR and NCR. This dis... rate caused a larger SV increase in NCR ... SHR while the TPR increase was cor...ndingly more pronounced in SHR (see Fig. 1) ... also be seen from Fig. 1 that the frequen...ponse curve for the MAP increase tended to ...latively steeper in NCR than in SHR so that ... the maximal blood increase was reached at a ...ly lower discharge frequency in NCR, in other ...s, for SHR the flatter CO response curve ...nates over the steeper TPR one when it comes ... net MAP effects.

...mmarize Upon graded splanchnic vasocon... ...or fibre stimulation SHR responded with ... pronounced SV increases than NCR both in ...ute and relative terms reflecting a relative ...reactivity concerning the effects on stroke ...me caused by capacitance vessel responses In ...rast, the SHR splanchnic resistance vessels ...ayed a 'hyperreactivity' with respect to their ...onses to graded vasoconstrictor fibre stimula... evidently mainly reflecting the hemodynamic ...cts of the relative wall thickening present in ...anic precapillary parts of hypertensive car...vascular systems.

...CUSSION

...ording to numerous studies (for ref see Folkow ... Julius & Esler 1976) neurogenic mechanisms ...ear to be of great importance for the initiation of ...e common variants of primary hypertension in ...as well as in rats, particularly in SHR, though ...re is much to indicate that the sustained re... increase in the established phase of hypertension is dominated by mechanisms of a structural background (Folkow et al. 1973)

The present experiments were performed to explore the interactions between structural cardiovascular changes and neurogenic influences on systemic resistance and capacitance vessels in SHR. The effects on MAP TPR and CO were followed in anesthetized SHR and NCR where all the splanchnic vasoconstrictor fibres were first cut and then stimulated at increasing rates, covering the physiological range of discharge up to include also clearly supraphysiological frequencies. Since the adrenal glands were eliminated, the resistance increases upon splanchnic nerve stimulation directly reflect the strictly neurogenic effects in the combined gastrointestinal-renal-hepatic circuits, expressed in terms of their effects on TPR and on MAP The corresponding capacitance vessel constriction of the splanchnic region will in this situation mainly be expressed as SV increases caused by the enhanced central blood volume and diastolic filling, because heart rate and myocardial inotropism remained constant thanks to pharmacological blockade Due to the Frank Starling relationship SV will then increase largely in proportion to the capacitance vascular constriction but it will also be influenced by the prevailing *diastolic compliance of the heart.*

This experimental approach will, however not provide information about the absolute regional changes in resistance and capacitance, even though the combined gastrointestinal-renal-hepatic circuits make out a major fraction of the systemic circulation in rats. Furthermore even if cardiovascular reflex excitability was greatly suppressed by using nembutal anesthesia (40–50 mg/kg b w) pressure induced compensatory reflex changes of resistance and capacitance in other circuits will tend to damp the impact of the splanchnic vascular adjustments on MAP CO and TPR.

In connection with the preparation MAP was reduced by 40% (75 mmHg) in SHR and by 30% (40 mmHg) in NCR. In SHR nearly half of this MAP fall was associated with the opening of the abdominal cavity while this procedure contributed with less than one fourth of the pressure fall in NCR. To a great extent this reflects how the abdominal, 'extravascular' pressure in rats is of great importance for avoiding pooling of blood in the splanchnic capacitance vessels. This, in combination with other elements are likely to contribute to the dras-

tic reduction in MAP after abdominal opening in SHR The remining MAP reduction some 40 mmHg in SHR and 30 mmHg in NCR was closely linked to adrenal gland denervation and splanchnic nerve section During the preparation the percentual fall in SV and CO was even greater for both SHR and NCR than their corresponding MAP reductions implying that TPR rose considerably even though the splanchnic nerves were cut

After completing the preparation stroke volume (SV) was larger in NCR than in SHR and SV also increased more steeply at low rates of splanchnic vasoconstrictor fibre stimulation in NCR (Fig 1) This can hardly be explained by a reduced sensitivity to nerve stimulation of the capacitance side of the vascular bed in SHR since considerable differences in SV between SHR and NCR occurred also at supramaximal stimulation frequencies The observed differences in SV increase upon nerve stimulation between SHR and NCR rather suggests a relative dysbalance in SHR between the low-pressure capacitance side and the hypertrophied left ventricle as also indicated by in vitro studies of left ventricular diastolic complicance in SHR and NCR (Hallbäck Isaksson & Noresson 1975 Noresson et al 1979) Even though there is evidence that also the systemic capacitance vessels exhibit a structurally reduced compliance in SHR hypertension (Simon 1976) this is evidently not enough to fully offset the hemodynamic consequences of the similarly reduced left ventricular compliance In SHR an increased left ventricular filling pressure seems to be more important for compensating the consequent Frank Starling shift than is a neurohormonally increased inotropism (Noresson et al 1979 Noresson Thorén & Hallback Nordlander 1979) It follows that the capacitance section in SHR would need a higher vasoconstrictor fibre activity than in NCR to maintain the same SV level at least when disturbances like blood loss reduced extravascular support (like the abdominal pressure) increased intrathoracic pressure etc interfere with strictly cardiovascular control mechanisms

In contrast, the neurogenic resistance increases are both in absolute and relative terms more powerful in SHR and the frequency-response curve for the neurogenic resistance control is here also steeper than in NCR despite the fact that the neurogenic constrictions in SHR faced much higher transmural vascular pressures This must be a reflection of the unspecific vascular hyperreactivity characterizing hypertensive precapillary [illegible] vessels when responding to both vasocon[illegible] and vasodilator agents and which is a mere [illegible] cal consequence of their structurally increased [illegible] (cf Folkow et al 1974) as also illustrated [illegible] precise morphometric and biophysical studies [illegible] in man (e g. Suwa & Takahashi 1971) and [illegible] (Mulvany & Halpern 1977) This accentuated [illegible] cular reactivity in SHR cannot be ascribed to [illegible] smooth muscle supersensitivity as such a [illegible] present could not explain the particularly [illegible] responses at high discharge rates. Then the supramaximal noradrenaline concentrations [illegible] reached in the narrow neuroeffector [illegible] characterizing resistance vessels (Johansson et al 1972, Folkow 1976).

As mentioned in the introduction [illegible] (1973) observed that higher frequencies [illegible] splanchnic stimulation were needed in SHR [illegible] NCR to restore MAP and it was concluded [illegible] also in established SHR hypertension the [illegible] MAP level is essentially due to an [illegible] sympathetic discharge to the resistance [illegible] Here the important capacitance vascular [illegible] was not considered but the present results [illegible] that it is indeed the increasing difficulties to [illegible] the capacitance low-pressure side to the [illegible] left ventricular compliance in SHR which [illegible] explains these results

In the present experiments it was, [illegible] shown that the rates of sympathetic [illegible] needed to restitute the MAP levels present [illegible] abdominal opening but before the surgical [illegible] ence with the splanchnic nerves, were almost [illegible] cisely the same in SHR and NCR i e 3-3[illegible] This is quite likely an overestimation for both [illegible] and NCR because of the inevitable gradual [illegible] ration of cardiovascular responsiveness along [illegible] the duration of the experiments but the [illegible] that SHR and NCR do not differ in this [illegible] The right panel illustrates how much more [illegible] ful the TPR increase would be in SHR than [illegible] at such equal discharge rates while the [illegible] true for the ability of the capacitance [illegible] enhance the filling of the left ventricle

It is thus also evident from the present [illegible] ments that the neurogenic capacitance [illegible] trol becomes increasingly compromized [illegible] its ability to adjust cardiac filling as left [illegible] lar hypertrophy gradually develops along [illegible] hypertension This chan[illegible]entuates [illegible]

on the vasoconstrictor fibre control of the low-pressure capacitance side. Therefore, y due to per se adequate adaptive structural es of the left heart and the systemic precapil- resistance vessels, the hypertensive cardio- lar system faces increasing problems to inte- the capacitance, pumping and resistance func into an efficient and economically function- pply system, tending to result in a relatively ed CO and an unproportionally enhanced nce resistance at a raised MAP level.

normalize such a situation by way of neuro- control mechanisms it would be necessary duce sympathetic discharge to the resistance ds and to enhance it to the capacitance side. It ubtful, however whether the bulbar centres heir reflex control systems really are capable such markedly differentiated patterns of pathetic discharge If not, there is also in this ct a tendency towards a vicious circle inherent hypertensive situation, apart from that due to ositive feedback interaction between function- ictatory influences and structural resistance el changes (Folkow Grimby & Thulesius). Whenever average MAP elevations trigger iac hypertrophy this calls for an increased fill- pressure to maintain stroke volume but the led increase of sympathetic activity then ac mates the resistance elevation as well and hence MAP level, and so on

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As mentioned in the introduction, Iriuchijima (1973) observed that higher frequencies of splanchnic stimulation were needed in SHR than NCR to restore MAP and it was concluded that also in established SHR hypertension the elevated MAP level is essentially due to an increased sympathetic discharge to the resistance vessels. Here the important capacitance vascular section was not considered but the present results suggest that it is indeed the increasing difficulties to match the capacitance low pressure side to the reduced left ventricular compliance in SHR which largely explains these results

In the present experiments it was however shown that the rates of sympathetic stimulation needed to restitute the MAP levels present after abdominal opening but before the surgical interference with the splanchnic nerves were almost precisely the same in SHR and NCR i e. 3-3. This is quite likely an overestimation for both SHR and NCR because of the inevitable gradual deterioration of cardiovascular responsiveness along the duration of the experiments, but the point is that SHR and NCR do not differ in this respect The right panel illustrates how much more powerful the TPR increase would be in SHR than in NCR at such equal discharge rates while the reverse is true for the ability of the capacitance vessels to enhance the filling of the left ventricle

It is thus also evident from the present experiments that the neurogenic capacitance vessel control becomes increasingly compromized concerning its ability to adjust cardiac filling as left ventricular hypertrophy gradually develops along with hypertension This change accentuates th

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al function in hypercalcemic dogs during hydropenia during saline infusion

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LINS, L. E. Renal function in hypercalcemic dogs during *hydropenia and during saline* infusion. Acta Physiol Scand 1979 106:177-186. Received 17 Nov 1978 ISSN 0001 6772. Department of Medicine, Renal Unit and the Urological Research Laboratory Karolinska Hospital, and the Department of Experimental Surgery Karolinska Institute Stockholm, Sweden

The effects of calcium-gluconate infusions on renal function were studied in unanesthetized dogs. Each dog was studied during hydropenia and saline infusion. Hypercalcemia, mean serum calcium 3.85 mmol/l (hydropenia) and 3.62 mmol/l (saline infusion), increased fractional excretion of sodium (C_{Na}/C_{In}), calcium (C_{Ca}/C_{In}) and magnesium (C_{Mg}/C_{In}). The increase was significantly higher in saline-expanded dogs than in hydropenic dogs. Fractional excretion of potassium (C_K/C_{In}) was increased in hydropenia but remained unchanged in saline-expanded animals. Fractional excretion of phosphate (C_P/C_{In}) was not consistently changed by hypercalcemia. *Fractional excretion of chloride* (C_{Cl}/C_{In}) was markedly increased in saline-expanded dogs but was not changed in hydropenia. Urine osmolality was reduced in hydropenic dogs but unchanged in saline-expanded dogs. In hydropenic as well as in saline-expanded dogs tubular reabsorption of solute-free water ($T^c_{H_2O}/C_{In}$) increased during the first hour of hypercalcemia. In hydropenic dogs hypercalcemia caused slight but significant decrease in blood pH, standard bicarbonate, and base excess. In hydropenic as well as in saline-expanded dogs glomerular filtration rate (C_{In}), renal plasma flow (C_{PAH}), and filtration fraction were unaffected.

Key words: Renal function, hypercalcemia, hydropenia, saline infusion

renal response to hypercalcemia has earlier studied in spontaneously occurring disease in nts and in a variety of animal experiments The istently observed alteration in renal function e loss of the ability to concentrate the urine ually (Epstein 1968) The effect of hyper emia on the glomerular filtration *rate (GFR)* ill controversial Chronic severe hypercal- ia is known to decrease GFR in both ani- l and humans (Epstein 1968), but the effect oderate hypercalcemia on GFR is uncertain In hypercalcemia induced by long-term para- oid hormone treatment caused a decrease in R (Carone et al. 1960 Engfeldt et al. 1962, Ben- 1978) In dogs treated in the same way for only r days the GFR was unchanged (Carone 1960). he induction of acute hypercalcemia, mostly by cium-gluconate or calcium-chloride infusions, given divergent results concerning the effects on GFR. In some studies hypercalcemia caused alterations in tubular function but no changes in GFR or renal blood flow (RBF) (Levitt et al. 1958, Leme & Wajchenberg 1972) In expts. with calcium gluconate Wallach & Carter (1961) found no consistent change in GFR until serum calcium reached a *level of 5 mmol/l However Edwards et al (1974)* found a significant decrease of GFR during acute hypercalcemia, serum calcium 3 95 mmol/l, in dogs The difference in results could be due to several factors. The origin and duration of hypercalcemia anesthesia, and state of hydration vary widely from one study to another Some experimental data *(Bank & Aynedjian 1965 Suki et al. 1969) suggest* different effects on GFR in hydropenia and in water diuresis In the present study standardised clearance techniques have been applied to evaluate the renal response to acute moderate hypercalcemia in unanesthetised dogs. We have studied the hy

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[illegible] Effects of acute hypercalcemia on renal function in seven dogs during hydropenia and during [illegible] infusion

[illegible]ntrol period represents the average of two 20-min periods, the calcium infusion and the post-infusion periods the [illegible] of three 20-min periods each. The results are expressed as mean ± 1 S.D. The *P* values represent significance of [illegible]nces versus control and were calculated by paired observations.

[illegible]		Control	Calcium infusion		Post-infusion	
[illegible] mmol/l	Hydropenia	[illegible].64±0.09	3.85±0.32	*P*<0.001	3.86±0.34	*P*<0.001
	Saline infusion	2.61±0.06	3.62±0.14	*P*<0.001	3.47±0.22	*P*<0.001
[illegible]/min	Hydropenia	0.18±0.05	0.61±0.10	*P*<0.001	0.38±0.06	*P*<0.001
	Saline infusion	1.52±1.10	1.49±0.34	n.s.	[illegible].43±1.19	n.s.
[illegible]l/min	Hydropenia	66±18	7[illegible]±21	[illegible]	65±17	n.s.
	Saline infusion	68±11	67±15	n.s.	67±15	n.s.
[illegible] ml/min	Hydropenia	221±72	238±82	n.s.	215±65	n.s.
	Saline infusion	203±51	198±48	n.s.	195±52	n.s.
[illegible]	Hydropenia	0.31±0.04	0.31±0.05	n.s.	0.31±0.05	n.s.
	Saline infusion	0.35±0.05	0.34±0.05	n.s.	0.35±0.04	n.s.
[illegible] mOsm/kg H_2O	Hydropenia	1 746±438	985±214	*P*<0.001	1 140±[illegible]47	*P*<0.001
	Saline infusion	479±405	541±104	n.s.	414±136	n.s.
C_{Na} %	Hydropenia	1.7±0.4	2.8±0.4	*P*<0.001	2.2±0.2	*P*<0.01
	Saline infusion	1.8±0.4	3.6±0.7	*P*<0.001	4.0±1.4	*P*<0.01
$/C_{In}$ %	Hydropenia	1.4±0.4	1.9±0.4	*P*<0.001	1.6±0.2	n.s.
	Saline infusion	−0.4±1.5	1.4±0.7	*P*<0.001	0.6±1.5	n.s.

[illegible]rterial oxygen tension (P_{O_2}) was determined with an [illegible] electrode (Radiometer, Copenhagen, Denmark). [illegible] carbon-dioxide tension (P_{CO_2}) and pH were de[illegible]ned by Astrup [illegible] technique (1956) with pH electrode [illegible]ometer, Copenhagen, Denmark). Base excess was [illegible]ined by the method of Siggaard-Andersen & Engel [illegible].

[illegible] hematocrit of heparinized blood samples was de[illegible]ned in [illegible] hematocrit centrifuge at 10 000 RPM for 5

All analyses were made in duplicate.

Calculations. The filtration fraction (FF) was calculated as GFR/RPP (C_{In}/C_{PAH}). Tubular reabsorption of solute-free water ($T^c_{H_2O}$) was calculated from the expression ($C_{osm}-V$) where V = urine flow in ml/min and C_{osm} = clearance of osmoles in ml/min. Fractional excre[illegible]tions of sodium (C_{Na}/C_{In}), potassium (C_K/C_{In}), calcium (C_{Ca}/C_{In}), magnesium (C_{Mg}/C_{In}), phosphate (C_P/C_{In}) and chloride (C_{Cl}/C_{In}) are calculated from total serum concentrations. Since only 65% of calcium and magnesium is

[illegible]le 3. Effects of acute hypercalcemia on renal [illegible]xcretion of electrolytes in seven dogs during hydropenia [illegible] during saline infusion

[illegible] control period represents the average of two 20-min periods, the calcium infusion and post-infusion periods the [illegible]age of three 20-min periods each. The results are expressed as mean ± 1 S.D. The *P* values express significance of [illegible]rences versus control and were calculated by paired observations.

		Control	Calcium infusion		Post-infusion	
$/C_{In}$ %	Hydropenia	0.3±0.2	0.9±0.[illegible]	*P*<0.001	0.4±0.1	n.s.
	Saline infusion	0.5±0.4	1.8±0.6	*P*<0.001	2.0±1.2	*P*<0.01
C_{In} %	Hydropenia	15.9±3.2	25.6[illegible]3.3	*P*<0.001	26.1±4.9	*P*<0.001
	Saline infusion	17.0±3.5	19.3±5.5	n.s.	22.6±5.5	[illegible]
$/C_{In}$ %	Hydropenia	0.1±0.1	3.3[illegible]0.8	*P*<0.001	2.7±0.7	*P*<0.001
	Saline infusion	0.1±0.2	4.4±1.3	*P*<0.001	4.8[illegible]1.8	*P*<0.001
$/C_{In}$ %	Hydropenia	3.4±2.7	8.0±2.3	*P*<0.001	7.3±1.6	*P*<0.001
	Saline infusion	3.3[illegible]2.8	10.3 [illegible] 3.8	*P*<0.001	11.6±3.3	*P*<0.001
	Hydropenia	11.2±6.9	9.1±4.7	n.s.	5.0±3.1	n.s.
	Saline infusion	11.0±6.9	8.6±5.3	n.s.	3.7±2.6	*P*<0.05
[illegible]		0.3±0.1	0.3±0.1	[illegible]	0 [illegible]±0.2	[illegible]
		0.5[illegible]0.3	1.4±0.6	*P*<0.01	2.6±1.8	*P* [illegible]

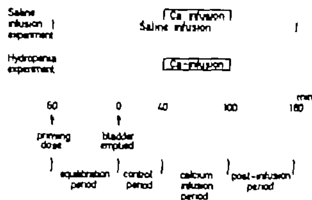


Fig 1 Basic time schedule for experiments

percalcemic effect on GFR renal plasma flow tubular reabsorption of solute free water the fractional excretion of electrolytes and acid-base balance in hydropenia as well as during saline infusion

MATERIAL AND METHODS

The experiments were performed on seven trained unanesthetised female mongrel dogs weighing 19– 4 kg. All animals were studied in hydropenia as well as during saline infusion For three weeks prior to the study the dogs were given a standard diet without meat supplying approximately 0 8 g of calcium and 0.4 g of phosphate per kg body weight per day At three weeks before the experiment a perineotomy was performed under general anesthesia and urine samples were obtained during the experiments by means of an infant-feeding tube size Fr 10 (Argyle® Sherwood Medical Industries Inc) The urethra was anesthetised with xylocain jelly prior to catheterization and the tube was kept in place with the aid of a ...

For infusions and blood sampling, cannulas were inserted into peripheral veins Blood samples were drawn at the mid-point of each clearance period. The length of the periods varied from 20–30 min. The bladder was emptied at the end of each period by air insufflation, vacuum suction and gentle pressure on the bladder To ensure complete emptying during hydropenia, the bladder was rinsed with 20 ml of sterile water at the end of each period Clearance periods were run while the dog was loosely restrained standing in the upright position.

Standard clearance techniques were used. GFR was measured as the clearance of inulin and RPF as the clearance of PAH both in ml/min. A prime dose of ... ml/kg b.wt. of a solution containing 85 mg/ml of inulin (Inutest®) and 30 mg/ml of para-aminohippurate (PAH Merck Sharp & Dohme) was given The solution was then infused with a motordriven syringe at a constant rate of 0.3 ml/min The plasma levels of Inutest and PAH were allowed to equilibrate for 60 min. Serum concentration of PAH did not exceed 190 µmol/l.

Analytical methods Inulin in serum and urine was determined spectrophotometrically by Heyrovsky's method (1956) and PAH was measured by Brun's method (1951) Serum and urine osmolality was determined by freezing point depression with a Knauer osmometer

Sodium and potassium concentrations in serum and urine were determined with a flame photometer (IL 143)

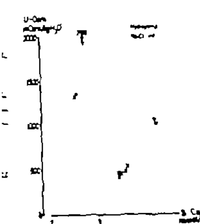
Chloride concentration in serum and urine was determined with the aid of a chloride titrometer (Aminco Cotlove, Silver Springs, Md Washington, D C, USA) Phosphate in serum and urine was determined colorimetrically by the method of Fiske & Subbarow (1925) Total calcium and magnesium concentrations in serum and urine were measured by atomic absorption spectrophotometry (Perkin-Elmer 403)

Serum albumin was determined spectrophotometrically by the method of Doumas et al. (1971)

Table 1 *Effects of saline infusion (control expts) on renal function and excretion of electrolytes in 7 dogs*

Period I represents the average of two 20-min periods periods II and III the average of three 20-min periods each. The results are expressed as mean ±1 S D The *P* values represent significance of differences in periods II and III versus period I and were calculated by paired observations

	Period I 0–40 min	Period II 40–100 min		Period III 100–160 min	
S-Ca, mmol/l	.56±0.10	.53±0 10	n s	49±0 09	$P<0.01$
V ml/min	0.78±0 61	1 38±1 16	$P<0.05$	1.57±0.95	$P<0.05$
C_{in} ml/min	75±19	74±20	n s.	76± 1	n s
C_{PAH} ml/min	227±57	12±34	n s	219±51	n s
FF	0 33±0 04	0.35±0 05	n s	0.35±0.08	n s
U_{osm} mOsm/kg H_2O	896±759	775±750	$P<0.05$	605±674	n.s
C_{osm}/C_{in} %	1 7±0.7	1 7±0 4	n s	1 7±0 4	n s
$T^c_{H_2O}/C_{in}$ %	0.4±1 1	−0.6±2.1	$P<0.05$	−0.7±1 8	n s.
C_{Na}/C_{in} %	0.5±0.6	0.5±0.5	n s	0.7±0 6	$P<0.05$
C_K/C_{in} %	13.3±5 2	11 4±4 7	n s.	10 4±3 2	n s.
C_{Ca}/C_{in} %	0.–±0 4	0 1±0 1	n.s.	0 1±0.1	n s
C_{Mg}/C_{in} %	2 4±1 2	2.4±1 1	n.s	2.4±1	n.s
C_P/C_{in} %	5.3±5 3	5 2±4 9	n s	6.3±5 5	n s
C_{Cl}/C_{in} %	0.5±0.5	0.5±0 2	n s.	0 7±0 4	n s.



3. Relationship between serum calcium and urine osmolality in hydropenic and saline-expanded dogs. Mean regressions are expressed by the equations $y = 3180 - 546x$ (hydropenia) and $y = 308 - 52x$ (saline infusion), $P < 0.01$

...a hematocrit were not altered by the saline infusion.

...nfusions of calcium gluconate raised serum calcium in hydropenic dogs from 2.68 to 3.85 mmol/l ($P < 0.001$) and in saline-expanded dogs from ...1 to 3.62 mmol/l ($P < 0.001$). Sustaining infusion ...calcium maintained the serum calcium at this ...d for 2 h.

...n spite of pronounced hypercalcemia, GFR, ...F and FF were unaffected (Table 2). During the ...ly phase of calcium infusion a slight but not ...ificant increase in GFR and RPF was seen in ...ne dogs. At the same time a tachycardia was ...ced. Urine flow increased in hydropenia from ...1 ml/min to maximally 0.61 ml/min but was unchanged during saline infusion (Table 2). In all ...pts. serum osmolality varied between 267 and 321 mOsm/kg H_2O. Urine osmolality was significantly ...duced ($P < 0.001$) in hydropenic dogs but unchanged in the saline-expanded animals (Table 2). ...actional osmolal excretion increased in all expts. ...able 2). In hydropenic as well as in saline-expanded dogs tubular reabsorption of solute-free water increased during the first hour of hypercalcemia ($P < 0.001$); in the second hour, the post-infusion period, it was unchanged compared with before calcium infusion (Table 2).

Fractional excretion of sodium, calcium, and magnesium increased significantly in hydropenic dogs as well as in saline-expanded animals (Table 3). As fractional excretions of calcium and

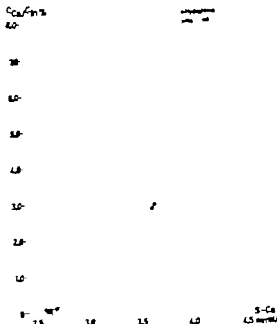


Fig. 4. Relationship between serum calcium and fractional excretion of calcium (C_{Ca}/C_{In}) in hydropenic and saline-expanded dogs. Mean regressions are expressed by the equations $y = -5.8 + 2.3x$ (hydropenia) and $y = -11.6 + 4.6x$ (saline infusion), $P < 0.001$

magnesium were calculated from total serum concentrations of calcium and magnesium, these figures are only about 65% of their actual values. Fractional excretion of potassium increased in hydropenic dogs but remained unchanged in saline-expanded animals (Table 3). Fractional excretion of phosphate was significantly reduced only in saline-expanded dogs during the post-infusion period (Table 3) but in hydropenic dogs as well as in saline-expanded dogs an increase in serum calcium concentration was followed by a decreased fractional excretion of phosphate (Fig. 2). Fractional excretion of chloride was not reduced in hydropenia. In saline infusion, however, fractional excretion of chlorides were increased (Table 3).

The concentration of PAH in urine was significantly higher in hydropenic dogs than in saline-expanded dogs. In the control periods concentrations of PAH were 28.6±9.5 versus 16.1±11.. mmol/l ($P < 0.05$), in the calcium-infusion periods 20.5±6.4 versus 12.5±2.7 mmol/l ($P < 0.01$) and in the post-infusion periods 22.7±6.4 versus 10.1±3.7 mmol/l ($P < 0.001$).

Hypercalcemia caused a slight but highly sig-

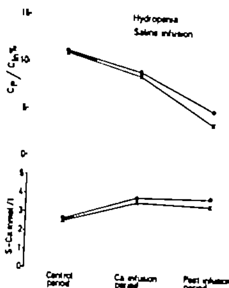


Fig 2 Serum calcium concentration and fractional excretion of phosphate (mean values) in hydropenic and saline-expanded dogs during control and experimental periods

ultrafiltrable the actual fractional excretion of these electrolytes is 35% higher

Standard statistical methods were used including paired observations and Student's *t* test All values are given as means ± standard deviation

Experimental procedure All animals were deprived of food and water for 18 h prior to each expt The design of the study is shown in Fig. 1 The saline solution contained 75 mmol NaCl per litre and the infusion rate was 0.. ml/kg/min Hence a volume corresponding to 1% of the body weight was given every 50 min. This kind of infusion has earlier been shown not to change GFR, FF or serum-sodium concentration (Aperia & Broberger 19..). At 1–3 weeks prior to the calcium-infusion study all animals passed a control study with saline infusion for .. min

Hypercalcemia was induced by the administration of a priming dose of calcium gluconate i.v (0.3 mmol Ca^{2+} per kg b wt.) followed by calcium infusion (5 μmol Ca^{2+} per kg b wt per minute) during 1 h corresponding to a total dose of 0 6 mmol Ca^{2+} per kg b.w.t. In each dog the effect of calcium infusion on renal function was studied in hydropenia as well as during saline infusion. More than .. weeks elapsed between the hydropenia expt. and the saline-infusion expt

RESULTS

During saline infusion serum calcium decreased from 2 56 to 2 49 mmol/l ($P<0\ 01$) (Table I). Urine flow increased from 0 78 to 1.57 ml/min. GFR RPF and FF were unchanged Fractional osmolal excretion was not altered but a small decrease was seen in urine osmolality and in tubular reabsorption of solute free water Fractional excretions of potassium calcium magnesium phosphate and chloride were not changed by saline infusion. At the end of the infusion fractional excretion of sodium was slightly increased (Table I) Venous pH standard bicarbonate base excess oxygen tension, carbon dioxide tension oxygen saturation serum albumin

Table 4 *Effects of acute hypercalcemia on pH standard bicarbonate base excess and hematocrit in seven dogs during hydropenia and during saline infusion*

The control period represents the average of two 20-min periods the calcium infusion and post-infusion periods the average of three 20-min periods each The results are expressed as mean ± 1 S D The *P* values express significant differences versus control and were calculated by paired observations

		Control	Calcium infusion		Post-infusion	
pH	Hydropenia	7.37±0 02	7 34±0.02	P<0 001	7 33±0.03	P<0 001
	Saline infusion	7.36±0 03	7 34±0 04	n s	7 34±0 04	n.s.
Standard bicarbonate mmol/l	Hydropenia	21±1	20±1	P<0 01	20±1	P<0 01
	Saline infusion	1±1	20±1	P<0 05	20±2	P<0.01
Base excess mmol/l	Hydropenia	−4±1	−5±1	P<0 01	−5±1	P<0 01
	Saline infusion	−3±1	−4±1	P<0 01	−5±	P<0 05
P_{CO_2} mmHg	Hydropenia	37±4	38±4	n s	39±4	n.s
	Saline infusion	38±3	39±4	n	38±3	n s.
P_{O_2} mmHg	Hydropenia	55±10	60±9	n	57±10	n.s.
	Saline infusion	60±13	61±11	n s	59±13	n.s.
Oxygen saturation, %	Hydropenia	85±8	86±7	n.s	85±8	n.s.
	Saline infusion	86±9	86±6	n s	85±9	n s.
Hematocrit %	Hydropenia	38±4	37±4	P<0 05	37±4	n.s.
	Saline infusion	39±4	37±5	P<0.05	36±5	P<0 01

doses of vitamin D_3 (Zawistowski & Roszkowski 1974). In dogs, the administration of parathyroid extract produced structural alterations localised mainly to the ascending limb of Henle, the distal convoluted tubules, and the collecting system. In these animals the decrease of GFR was related to the degree of intratubular obstruction (Carone et al. 1960). In studies by Engfeldt et al. (1962) the correlation between functional and structural changes was poor. They discussed whether the decrease in GFR and RPF was due to the moderate hypercalcaemia or to a direct effect on the kidney of the parathyroid extract.

In acute hypercalcaemia, mostly induced by calcium-chloride or calcium-gluconate infusions, the effect on GFR and RPF is controversial. At high serum-calcium levels, above 5 mmol/l, GFR and RPF are consistently depressed (Chen & Neuman 1955, Edwards et al. 1974, Tarkovics et al. 1974, Humes et al. 1976). Wallach & Carter (1961), studying hydrated dogs, found that when the plasma concentration of calcium rose to between 3.75 and 5.25 mmol/l either GFR or RPF decreased in half the dogs. Marked impairment of GFR and RPF occurred at serum-calcium levels above 5.25 mmol/l. In dogs which had been allowed free access to water the elevation of serum calcium from 2.45 to 3.95 mmol/l produced a slight but significant fall in GFR (Edwards et al. 1974). Lassiter et al. (1963) infused calcium chloride into the renal artery of one kidney in hydropenic dogs. The opposite kidney served as control. In both kidneys GFR and RPF decreased moderately when serum calcium increased from 2.30 to 2.75 mmol/l. The effect was greater in the experimental kidney. There was also a significant negative correlation between relative changes in serum calcium and GFR. In man and monkeys Levitt et al. (1958) found no changes in GFR or RPF when serum calcium increased from 2.35 to maximally 5.35 mmol/l.

Disregarding different serum-calcium levels, the divergent renal response to hypercalcaemia could be explained by differences in the experimental models, i.e. by various state of hydration. Both in animals and in man volume expansion has been shown to increase GFR (Wesson et al. 1950, Agnata et al. 1973). In calcium-infusion experiments with simultaneous volume expansion the latter could mask a depression of GFR induced by hypercalcaemia. Acute hypercalcemia, serum calcium 4.25 mmol/l, induced by calcium-lactate infusions reduced GFR by 30 to 60% in hydropenic dogs (Suki et al. 1969). In dogs in water diuresis there was no consistent fall in GFR. The reason for this difference is unknown but Suki et al. suggest that it could be related to a high renal content of calcium during hydropenia.

In the present study dogs were carefully selected and trained to stand repeated experiments. Thus, each dog was investigated in a hydropenic and in a hydrated state. Individual differences between different animals could thus be eliminated when the two groups of experiments were compared. In order not to influence GFR the dogs were kept on a standard diet. In spite of serum-calcium levels exceeding 3.50 mmol/l for 2 h and a fall in parathyroid-hormone concentration, shown by a decreased fractional excretion of phosphate (Fig. 7) GFR, RPF and FF were not affected in hydropenia or saline-expansion experiments. Unlike Suki et al. (1969) we could not find that the state of hydration altered the renal response to hypercalcemia.

During calcium infusion in hydropenic dogs urine osmolality decreased but the urine remained hypertonic. These results are in agreement with the reports of Brunette et al. (1974). In saline-infused animals urine osmolality was unaffected. Suki et al. (1969) found that tubular reabsorption of solute-free water was consistently reduced in hydropenic hypercalcemic dogs. In hydrated dogs free-water clearance was reduced. Suki et al. concluded that a defect of sodium transport in the loop of Henle was the probable explanation. In the present study fractional reabsorption of solute-free water was increased during the early phase of hypercalcemia in hydropenic and hydrated dogs. The hypercalcemia produced a positive fractional reabsorption of solute-free water in the saline-expanded dogs. These results are in contrast to those of Beck et al. (1959) who found fractional reabsorption of solute-free water to be slightly decreased in hypercalcemic dogs undergoing mannitol diuresis. Brunette et al. (1974) in agreement with Beck, found that tubular reabsorption of solute-free water decreased when calcium chloride was infused into the renal artery. They found a redistribution of the renal blood flow to the medullary regions of the kidney possibly responsible for the decreased cortico-medullary osmotic gradient and the hyposthenuria.

In spite of a moderate increase in fractional

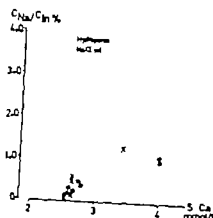


Fig 5 Relationship between serum calcium and fractional excretion of sodium (C_{Na}/C_{In}) in hydropenic and saline expanded dogs Mean regressions are expressed by the equations $y=-0\ 79+0\ 23x$ (hydropenia) and $y=-3\ 2+1\ 4x$ (saline infusion), $P<0\ 001$

nificant decrease in pH in hydropenic dogs In the saline-expanded dogs the decrease was not significant (Table 4) This tendency towards acidosis was verified by a decrease in standard bicarbonate and base excess in hydropenic and saline-expanded dogs (Table 4) P_{CO_2} P_{O_2} and oxygen saturation were unchanged in all expts (Table 4) Serum albumin changed by less than 10% in all expts Hematocrit decreased in hydropenic as well as saline-infused dogs during the calcium infusion A further decrease was found in the saline infused dogs during the post infusion period (Table 4)

Regression analysis Analysis were performed to find out whether the renal effects of hypercalcemia differed between hydropenic and saline-expanded dogs at comparable serum-calcium levels

The changes in GFR, RPF FF and fractional reabsorption of solute-free water were similar in hydropenia and saline infusion Urine osmolality was reduced in hydropenia but unchanged in the saline-iffusion experiments ($P<0\ 01$) (Fig 3) Fractional excretions of sodium calcium and magnesium were more pronounced in saline infused than in hydropenic dogs ($P<0.001$) (see Fig 4 and 5) Fractional excretion of chloride expressed as a function of fractional excretion of sodium was significantly higher in saline-expanded dogs than in hydropenic dogs (Fig 6) In hydrated dogs hypercalcemia markedly increased fractional excretions of sodium and chloride In hydropenic dogs minor changes in fractional excretion of sodium were found Fractional excretion of potassium was higher in hydropenic than in saline-expanded dogs ($P<0.05$) The effect of hypercalcemia on fractional excretion of phosphate did not differ between hydropenia and saline infusion expts.

In spite of a significant fall in blood pH in hydropenic dogs which was not found in saline-expanded animals (Table 4) there was no significant difference between the groups when regression analysis was used

The effects of hypercalcemia on standard bicarbonate base excess P_{CO_2} P_{O_2} and oxygen saturation were similar in hydropenic and saline expanded dogs

DISCUSSION

The mechanisms by which hypercalcemia affects the renal function have not been made fully clear Chronic hypercalcemia causes structural changes in the kidney such as tubular epithelial degeneration calcification and necrosis (Epstein et al. 1958 Carone et al 1960 Duffy et al. 1971) At the level of ultrastructure it appears that large doses of vitamin D cause increased uptake of calcium by the proximal tubules This is followed by morphologic changes of mitochondrial structure (Scarpelli 1965) Marked thickening and deposition of calcium salts in the basement membranes of the glomerular capillaries which might have an effect on glomerular filtration have been observed in rats treated with

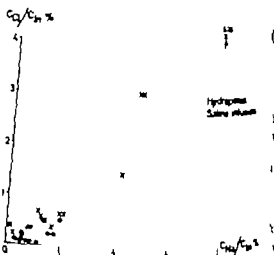


Fig 6 Relationship between fractional excretion of sodium (C_{Na}/C_{In}) and fractional excretion of chloride (C_{Cl}/C_{In}) in hydropenic and saline-expanded dogs. Mean regressions are expressed by the equations $y=0$ [illegible] $0.12x$ (hydropenia) and $y=0\ 04+1\ 03x$ (saline infusion) $P<0\ 01$

...een the two regimes with respect to the effects ...id-base balance.

...spite of the rapid increase in serum calcium ...nital tachycardia P_{O_2}, P_{O_2} and oxygen sat...ion were unchanged in all expts. The consistent ...ase in hematocrit in saline-infused dogs was ...ably due to a combination of blood loss and ...ease ...olume expansion

...study was supported by grants from the Swedish ...al Research Council (B76-19X-04798-01) and ...ska Institute, Stockholm. We are indebted to ...ne AB for the generous supply of calcium gluconate.

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reabsorption of solute free water the presence of decreased urine osmolality which we found in hydropenic dogs suggests an impairment in the renal concentrating capacity in acute hypercalcemia The decrease in fractional reabsorption of solute free water in saline-expanded dogs when fractional osmolal clearance exceeded 4 ml/min also indicate an impaired concentrating ability In the present study the increase in fractional osmolal clearance however was too small to allow detailed studies on the tubular reabsorption of solute free water and the concentrating ability

The discrepancies between our results and those of others concerning the effects of calcium on GFR RPF and urine concentrating ability could be due to the fact that we have used conscious dogs throughout and others mainly anesthetised dogs Increasing experimental evidence points to the important role of prostaglandins in regulating the renal circulation and Swain et al (1975) have recently shown that indomethacin a potent inhibitor of prostaglandin synthesis caused a reduction in renal blood flow in anesthetised dogs In conscious dogs on the other hand indomethacin did not alter renal blood flow Thus prostaglandins seem to play only a minor role in the control of the renal circulation in conscious animals but to be of greater importance in anesthetised animals

The fractional excretion of sodium calcium and magnesium increased in hydropenia as well as in the saline infusion experiments This is in agreement with earlier reports (Wallach & Carter 1961) The increased fractional excretion of sodium suggests a diminished reabsorption of sodium in hydropenic as well as in saline-expanded dogs The concurrent reduction of the concentrating ability indicates that the changes in sodium reabsorption are located in the ascending limb of the loop of Henle Excretions of sodium calcium and magnesium were however significantly higher in saline experiments than in hydropenia This difference was attributed to the hypercalcemia, since the fractional excretions of sodium calcium and magnesium were unaffected by the saline infusion in the control experiments (Table 1) This suggests that even hypotonic saline could influence the effect of calcium on the tubular reabsorption of other electrolytes The fractional excretion of potassium increased in hydropenic dogs which is in agreement with the reports of Wallach & Carter (1961) who found an increased excretion of potassium after intravenous infusion of calcium chloride In stop-flow studies by Sa[illegible] al (1960) fractional excretion of potass[illegible] creased in dogs after calcium loading indica[illegible] duced reabsorption and/or augmented secre[illegible] potassium It cannot be stated whether the [illegible] action between calcium and potassium o[illegible] throughout the length of the nephron or pred[illegible] antly in proximal or distal segments. It is po[illegible] that the marked increase in fractional excre[illegible] potassium in hydropenic dogs (Table 3) wou[illegible] caused by an increased delivery of sodium t[illegible] distal tubules and an increased exchange of so[illegible] for potassium

Fractional excretion of phosphate wa[illegible] significantly changed in the hydropenic dogs [illegible] present study Similar results have been rep[illegible] by Wallach & Carter (1961) in dogs and Harris [illegible] (1977) in rats In contrast to these results, Lav[illegible] & Pullman (1963) could demonstrate a decrea[illegible] phosphate excretion along with a decrease in [illegible] when calcium chloride was infused into the r[illegible] artery in dogs Since a decrease in phosphate ex[illegible] tion can be secondary either to a fall in fil[illegible] phosphate alone or to a fall in filtered phosp[illegible] plus a rise in net tubular reabsorption of phosph[illegible] the absence of a decrease in GFR in the pre[illegible] study could explain the absence of a signific[illegible] decrease of phosphate excretion Fractional ex[illegible] tion of chloride was significantly increased [illegible] saline infused dogs and this increase was par[illegible] to an increase of fractional excretion of sod[illegible] (Fig 5) In hydropenic animals at the same ser[illegible] calcium level fractional excretion of chloride [illegible] unaffected The constantly low fractional excret[illegible] of chloride in hydropenic dogs is explained by [illegible] presence of high concentrations of PAH in [illegible] urine The negative para aminohippurate ion [illegible] the anion gap in hydropenic dogs which leads to [illegible] low excretion of chloride

In man hypercalcemia produced by acute inf[illegible] sions of calcium is known to stimulate secretion [illegible] acid by the kidney (Richet et al 1963) In do[illegible] calcium infusions decreased urine pH and increase[illegible] the excretion of hydrogen and ammonium ion[illegible] without any changes in arterial blood pH or in con[illegible] centration of bicarbonate (Wallach & Carter 1961) In our study calcium infusion induced a slight b[illegible] consistent decrease of blood pH standard bicarbo[illegible] nate and base excess Although there were n[illegible] significant changes in blood pH in saline infused animals regression analyses showed no difference

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effects of changes in the carotid sinus baroreceptor vity on splanchnic blood flow in anesthetized man

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TYDÉN G., SAMNEGÅRD H & THULIN L. The effects of changes in the carotid sinus baroreceptor activity on splanchnic blood flow in anesthetized man. Acta Physiol Scand 1979 106: 187–189. Received 20 Nov 1978. Department of Surgery Karolinska Institutet, Serafimer Hospital, Stockholm, Sweden.

In 9 patients being subjected to abdominal surgery electromagnetic blood flow measurements were obtained from the hepatic, mesenteric and iliac beds while the carotid sinus baroreceptors were stimulated by carotid sinus massage. Carotid sinus stimulation produced an average maximum decrease in mean arterial pressure of 21%. Hepatic and mesenteric blood flows decreased by 15% and calculated vascular resistances were not significantly changed in these vascular beds. Iliac blood flow on the other hand, showed slight increase and iliac vascular resistance was decreased by 29%. It is concluded that the splanchnic vascular bed is of less importance in the carotid sinus baroreflex control of systemic arterial pressure in anesthetized man.

e the demonstration of the baroreceptor reflex tion of the carotid sinuses more than 50 years knowledge about the physiology of the reflex trol of circulation has accumulated at an ever easing rate. It is now wellknown that activation n carotid sinus stretchreceptors reflexly reduce rial pressure by decreasing total peripheral resistance and cardiac output. In man, the reduction vascular resistance seems to be predominant le changes in cardiac output are of less importance (Tydén 1977). It is still uncertain, however whether reflexly induced alterations in vascular resistance are confined to the skeletal muscle l or whether other vascular beds such as the anchnic bed, are involved. Information obtained n experiments in animals are conflicting. Thus, otid sinus baroreceptor stimulation has been own to have no (Löfving 1961 Resnicoff et al. 69), moderate (Vatner et al. 1970, Vatner Franklin & Braunwald 1971 Hainsworth & Karim 1976) d pronounced (Greenway Lawson & Mellander 67 Bond & Green 1969 Carneiro & Donald 1977) ects on splanchnic vascular resistance. In man upright posture has been demonstrated to evoke increase in hepatic vascular resistance (Culbertson et al. 1951) while hemorrhage of 15–20% of the total blood volume has been found to be without effect on total splanchnic vascular resistance (Price et al. 1966) Rowell et al (1972) on the other hand has found clearcut increments in splanchnic resistance in man during peripheral pooling of blood as induced by lower body negative pressure.

The present experiments were designed to further investigate the involvement of the splanchnic vascular bed in the carotid sinus reflex in man. Electromagnetic blood flow measurements were therefore performed in the hepatic, mesenteric and iliac beds in patients subjected to abdominal surgery while the carotid sinus baroreceptors were stimulated by carotid sinus massage.

MATERIAL AND METHODS

Nine patients with mean age of 68 years (range 48–77) served as subjects. They were all subjected to abdominal surgery for carcinoma in the splanchnic area. The investigation plan had been approved by the Ethical Committee of the Serafimer Hospital and the informed consent had been obtained from all subjects.

Anesthesia was induced with thiopentone and intubation was performed under suxamethonium. Intermittent posi-

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...nic vascular resistance during a 75° head-up ...man. In both these experiments arterial mean ...ulsatile pressure were decreased and the caro... ...inus baroreceptors may therefore have been ...ed. Nevertheless, these experiments allow no ...usions to be drawn regarding the receptors ...nsible for splanchnic vasoconstriction since ...roreceptors i.e. aortic and carotid sinus plus ...intrathoracic low-pressure baroreceptors were ...lated by lower body negative pressure as well ... head-up tilt. In the present experiments, on ...ther hand, only the carotid sinus baroreceptors ... stimulated. Since no significant alterations in ...chnic resistance occurred it must therefore be ...uded either that the splanchnic bed was not ...ved in the carotid sinus reflex or that the ...id sinus reflex was inactivated by surgical ...a or anesthesia (cf. Vatner & Braunwald 1975) ...ually that the reduction in arterial pressure ...used by the carotid sinus reflex was merely a ... of a reduction in cardiac output with no alt... ...in total peripheral resistance. The latter ex... ...ation seems unlikely since it has previously been ...n that the buffering function of the carotid ... baroreceptors in man is predominantly a re... ...f alterations in total peripheral resistance (Ty... ...977), changes in cardiac output being of minor ...rtance. Furthermore, the fact that carotid ... massage evoked a drastic reduction in iliac ...ular resistance indicates that changes in periph... ...resistance did occur in some vascular beds ... or anesthesia and surgical trauma both may ... the responses of the cardiovascular system to ...receptor stimulation. However since carotid ...pulation did evoke a considerable reduction in ...ial pressure in the present experiments the ...tid sinus reflex must have been intact. It seems ...able therefore that the splanchnic bed is not ...lved in the carotid sinus baroreflex control of ...emic arterial pressure in anesthetized humans.

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tive-pressure ventilation with nitrous oxide and oxygen and increments of fentanyl diazepam and pancuronium were given to maintain anesthesia.

Arterial blood flow measurements were obtained by electromagnetic blood flowmetry utilizing a three channel square wave electromagnetic blood flowmeter (Nycotron) In each subject arterial blood flow recordings were simultaneously obtained from 1–3 of the following vessels viz. the common hepatic artery the gastroduodenal artery the iliocolic artery the left colic artery and the external iliac artery During dissection of vessels for measurement special care was taken to preserve the continuity of the perivascular nerves. Zero flow was obtained by means of an occlusion loop placed distal to the flow probe

Intraarterial pressure was obtained from the radial artery by means of a strain gauge manometer Mean arterial pressure was electronically derived from the arterial pressure recordings.

Recordings All signals were continuously displayed and recorded on a multichannel Grass polygraph.

Statistics The statistical significance of differences between mean values were evaluated by applying the *t*-test to the intraindividual differences (cf Fisher 1948)

Procedure All measurements were obtained immediately following laparotomy before any surgery of the prevailing carcinoma had commenced When accurate recordings of blood flows and arterial pressure were obtained the carotid sinus stretchreceptors were stimulated by bilateral carotid sinus massage When mean arterial pressure had dropped by approximately 20% the carotid sinus massage was discontinued and arterial pressure was allowed to return to normal In every patient this procedure was performed at least twice

RESULTS

Changes in arterial pressure blood flows and calculated vascular resistances evoked by carotid sinus massage are given in Table 1 as percentage changes from control values

Arterial pressure Carotid sinus massage produced an average maximum decrease in mean arterial pressure of 21% from control (from 121 mmHg to 96 mmHg) Pressure began to fall immediately following the onset of carotid stimulation and the desired maximum decrease (−20%) was reached within 30 s. When the carotid sinus massage was discontinued pressure returned to the prestimulation level within one minute

Splanchnic blood flow Carotid sinus massage resulted in equal reductions of the blood flows in all splanchnic vessels studied i e the common hepatic artery the gastroduodenal artery and the iliocolic and the left colic arteries The reductions were of the same magnitude (average 15% Table 1) as the reductions in arterial pressure Calculated

Table 1 *Effect of carotid sinus massage on mean arterial pressure regional blood flow and vascular resistances expressed as percentage changes from control values*

	Mean arterial pressure	Blood flow	Vascular resistance
Common hepatic artery ($n=9$)	−20%***	−15%	−49[illegible]
Gastroduodenal artery ($n=3$)	−15%	−14%**	1[illegible]
Iliocolic artery ($n=7$)	−[illegible]%	−17%	49
Left colic artery ($n=4$)	−23%	−15%***	−4[illegible]
External iliac artery ($n=6$)	−23%	+5% n.s.	3[illegible]

$P<0.001$ $P<0.01$ $P<0.05$ n.s. $P>0.05$

vascular resistances were therefore not signi changed

Iliac blood flow In contrast to the spl blood flow carotid sinus massage evoked a increase in the blood flow in the external iliac although arterial pressure was decreased by Calculated iliac vascular resistance was th decreased by 29%

DISCUSSION

The major finding of this study was that sinus stimulation had only minor eff splanchnic vascular resistance although reductions occurred in arterial blood pres iliac vascular resistance. It seems that on previous studies have been performed co the participation of the splanchnic bed in th sinus reflex in man Price et al (1966) fo splanchnic vascular capacitance was drast duced in man during hemorrhage while sp resistance was unchanged However in t periments the hemorrhage was not accomp reductions in arterial pulse pressure or me sure and there were no changes in heart rat peripheral resistance It seems unlikely that the carotid sinus reflex was involved al (1972) on the other hand utilizing low negative pressure to induce peripheral p blood in man noticed a considerable in splanchnic vascular resistance Likewi bertsson et al (1951) found a signific

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aphylaxis in the monkey hemodynamics and od flow distribution

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Aggregate anaphylaxis was induced in eight ovalbumin-sensitized monkeys (Macaca irus). Hemodynamics, blood flow distribution and myocardial performance were studied. Following challenge, severe circulatory shock developed. Systemic arterial and left atrial pressures decreased and pulmonary arterial and right atrial pressures increased. There was tenfold increase in pulmonary ascular resistance and cardiac output was markedly reduced (−75%). A redistribution of the blood flow to vital organs (brain, heart and liver) occurred, at the expense of flow to other regions (muscles, kidneys, pancreas and spleen). There was also a redistribution of the blood flow within the myocardium, resulting in an unchanged right ventricular blood flow despite decrease in total myocardial blood flow Right ventricular stroke work was reduced in spite of high filling pressures, whereas the decrease in left ventricular stroke work coincided with low filling pressures. It is concluded that the initial main cause of the low outflow state was an increased resistance in the pulmonary circulation followed by acute right heart failure.

Key ords. Anaphylaxis, shock, microspheres, myocardial blood flow pulmonary vascular resistance, monkey

siderable interspecies variations in the patho-
siological manifestation of acute anaphylactic
tions have been demonstrated. Respiratory
ptoms predominate in the guinea pig, whereas
ations in the pulmonary circulation are more
ounced in the rabbit, and in the dog congestion
e liver is a prominent feature (Becker & Austen
5). Thus, to be able to refer the results obtained
imal experiments to man, the choice of animal
cies is important. The monkey would seem to be
itable animal for experimental anaphylactic in-
tigations (Feinberg & Feinberg 1971), because of
close anatomical, physiological and immunologi-
relationship to man. The present study is one of
eries undertaken to examine the early circula-
y respiratory and hematological changes in
phylactic shock in this animal. This particular
dy was designed to evaluate the hemodynamic
d peripheral blood flow changes in monkeys
acaca irus) subjected to aggregate anaphylaxis.
attempt was also made to study the myocardial
rformance during the reaction.

MATERIAL AND METHODS

Animals

Eight monkeys (Macaca irus) delivered from the National Bacteriological Laboratory (Stockholm, Sweden) were used. They were all males, weighing 2.1 to 4.3 kg.

Immunization

The animals were sensitized by 4 i.m. injections of 10 mg ovalbumin (Grade V Sigma Co.) (Smedegård et al. 1979). The priming dose was given in Freund' complete adjuvant and the three booster doses in Freund's incomplete adjuvant. The experiments were carried out approximately 10 weeks after the start of the immunization and 7–1 days after the last booster injection.

Anesthesia and surgical procedures

The animals were anesthetized with ketamine hydrochloride (Ketalar® Parke-Davis) given intramuscularly in dose of 15 mg/kg b.wt. Additional injections of 25 mg were given as required. The monkeys were placed on heated table to keep the body temperature constant (38°C) They breathed spontaneously during the expts. A tracheostomy was performed to assure clear airway The pulmonary artery was catheterized via the right internal jugular vein for recording of the pulmonary arterial pres-

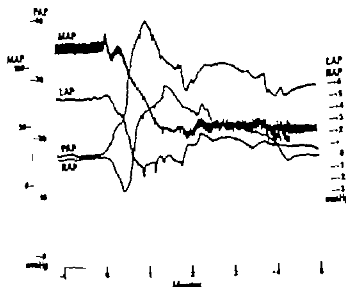


Fig. 2. The changes in mean arterial (MAP), left atrial (LAP), pulmonary arterial (PAP) and right atrial (RAP) pressures in one experiment during the first five min following challenge with ovalbumin at zero time. (1 mmHg = 0.133 kPa.)

the six monkeys. The ratio for left/right ventricular (LV/RV) was also determined.

... and statistics

... regional blood flows were calculated from the ...:

... reference flow · total injected activity / reference activity

... blood flow = reference flow · organ activity / reference activity

... peripheral (TPR), pulmonary vascular (PVR) and ... vascular resistance (CVR) were calculated from ... equations:

$$\frac{MAP - RAP\ (mmHg)}{CO\ (ml\ min^{-1})}$$

$$\frac{PAP - LAP\ (mmHg)}{CO\ (ml\ min^{-1})}$$

$$\frac{MAP - RAP\ (mmHg)}{Myocardial\ blood\ flow\ (ml\ min^{-1}\ 100\ g^{-1})}$$

... available oxygen for the left and right ventricles of the ... was calculated from:

... or RV blood flow (ml min^{-1} 100 g^{-1}) · Hb ... 1.34 (ml O_2 (g Hb)$^{-1}$) · SaO_2 (%) · 10^{-4}

left and right ventricular stroke work from the equations:

LVSW = (MAP − LAP) (mmHg) × stroke volume (ml) × 13.6 × 9.81 × 10^{-6}

and

RVSW = (PAP − RAP) (mmHg) × stroke volume (ml) × 13.6 × 9.81 × 10^{-6}

The LV and RV minute work were obtained by multiplication of LVSW and RVSW, respectively, by the heart rate. Paired Student's *t*-test was used for calculating *P* values.

RESULTS

Hemodynamic

The vascular pressure changes during the first 5 min after challenge in one experiment is shown in Fig. 2, and Fig. 3 shows the changes as means for the whole group of animals during 30 min. Challenge with ovalbumin resulted in a transient increase of MAP of 7±2 mmHg (mean±S.E.). This increase occurred in every animal and appeared after about 10 s. Following the initial increase in MAP (not shown in Fig. 3), there was a decrease which paralleled a decrease in LAP. Minimum values for MAP and LAP (47 and −2.4 mmHg, respectively) were recorded about 4 min after challenge. PAP increased and a transient decrease in RAP was observed. RAP reached a minimum value (−1.5

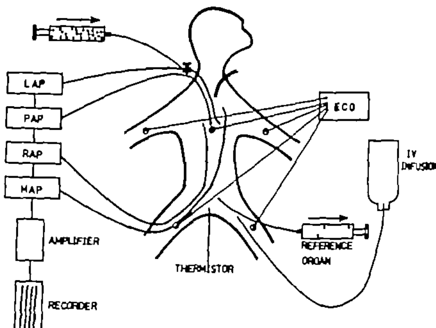


Fig 1 Experimental set up

sure (PAP) A catheter was inserted into the left atrium via the right common carotid artery and into the right atrium via the right femoral vein to measure left and right atrial pressures (LAP RAP) The mean arterial pressure (MAP) was recorded via a catheter in the aortic arch introduced via the right femoral artery The catheters were positioned fluoroscopically The left femoral artery was catheterized for blood flow measurements Through a catheter in the left femoral vein saline was infused (5 ml/kg/h) during the operative procedures to avoid dehydration Fig 1 shows the experimental set-up

Experimental procedures

The catheters for pressure recordings were attached to Statham transducers (P 23 AC P 23 BC) The mean pressures were recorded continuously on an UV oscillograph recorder (Ultralette ABEM Stockholm) Electrocardiograms (ECG) leads I II III and V were recorded continuously during the first 5 min after challenge and thereafter at regular intervals using a Mingograf 34 apparatus (Elema-Schonander) Cardiac output (CO) and regional blood flows were measured by a method originally described by Rudolph & Heymann (1967) and recently reviewed by Heymann et al (1977) Microspheres ($1-2 \times 10^6$) with a diameter of 15 μm labeled with ^{57}Co or ^{46}Co (Tracer Sephadex® Pharmacia Fine Chemicals Uppsala, Sweden) were suspended in 1 ml of monkey serum and placed on a vibrator just before injection to ensure a uniform suspension. The microspheres were injected into the left atrium over a period of 15–20 The arterial reference blood samples were taken from the catheter inserted into the left femoral artery The tip of the catheter was placed just below the aortic bifurcation and the proximal end was connected to a heparinized syringe attached to a withdrawal pump (Sage Instruments, model 355) The pump flow was calibrated and set at ~30 ml min (reference organ) It was started just before and stopped approximately 90 s after the commencement of the microsphere infusions

After induction of anesthesia and catheterization the animals were left to stabilize for 1 h before control measurements were made and the first set of microspheres was injected The animals were then challenged with a bolus injection of ovalbumin (70 mg/kg in ml of saline) through the catheter in the right femoral vein The second set of microspheres was injected 4 min after challenge when the shock seemed to be completely developed In five of the expts arterial blood samples were taken in connection with the CO determinations from the catheter in the aortic arch. The blood was analysed for oxygen saturation (SaO_2) and hemoglobin (Hb) on a CO-oxymeter (Instrumentation Laboratory Inc model 182) In the non-fatal cases the animals were observed for at least 30 min after challenge before they were killed At the end of the expt. the heart was excised Its surface was cleaned of blood and the cavities were swabbed with gauze Visible fat and large vessels were removed In six of the monkeys the heart was divided into right and left ventricular free walls. The base and the apex of the free wall of the left ventricle were further divided into endocardial epicardial and myocardial layers of approximately equal thickness The rest of the heart was divided into an appropriate number of pieces. The hearts of the other two monkeys were divided into a smaller number of pieces. The heart pieces together with pieces of tissues removed from other organs were weighed and their radioactivity was measured in a well-type gamma spectrometer (Packard Instruments model 5968) after which the blood flows were calculated Endocardial/epicardial blood flow ratios were calculated for the base and the apical part of the left ventricular free wall (LV base endo/epi, LV apex

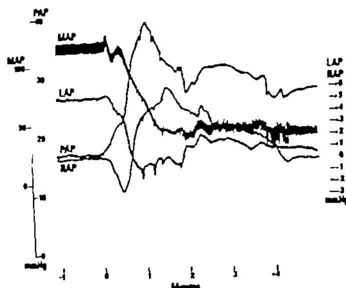


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MAP · RAP (mmHg) / CO (ml · min^{-1})

PAP · LAP (mmHg) / CO (ml · min^{-1})

MAP − RAP (mmHg) / Myocardial blood flow (ml · min^{-1} · 100 g^{-1})

...able oxygen for the left and right ventricles of the ...t was calculated from

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The vascular pressure changes during the first 5 min after challenge in one experiment is shown in Fig. 2, and Fig. 3 shows the changes as means for the whole group of animals during 30 min. Challenge with ovalbumin resulted in a transient increase of MAP of 7±2 mmHg (mean±S.E.). This increase occurred in every animal and appeared after about 10 s. Following the initial increase in MAP (not shown in Fig. 3), there was a decrease which paralleled a decrease in LAP. Minimum values for MAP and LAP (47 and −4.4 mmHg, respectively) were recorded about 4 min after challenge. PAP increased and a transient decrease in RAP was observed. RAP reached minimum value (−1.5

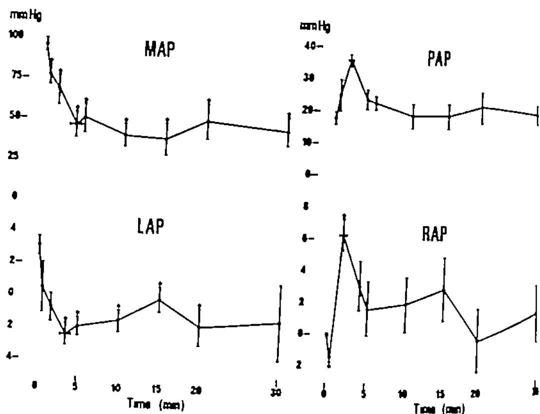


Fig 3 Mean values (± S E) for mean arterial (MAP $n=8$) left atrial (LAP $n=7$) pulmonary arterial PAP $n=$ right atrial (RAP $n=8$) pressures Zero time represents control measurements and challenge with ovalbumin Fo first 5 min values are plotted at the times when maximum pressure changes occurred The horizontal bars repre S E. for the mean time of maximum change indicates a statistically significant difference from controls ($P<0.05$) T of the animals died 15–20 min after challenge. (1 mmHg = 0 133 kPa)

mmHg) about 30 s after challenge When PAP increased further RAP showed a simultaneous increase PAP and RAP reached maximum values (36 2 and 6.3 mmHg respectively) in 2 min The maximum changes in PAP and RAP preceded the changes in LAP and MAP by about 2 min The decrease in CO recorded 4 min after challenge averaged 75 % (Table 1) The heart rate (HR) increased significantly but in some expts the increase was preceded by a short and transient period of bradycardia. TPR increased 1 5 times and there was a tenfold increase in PVR. The two animals with the largest CO reduction died 15 to 20 min after challenge Death was preceded by a gradual increase in LAP and RAP

ECG

The observed ECG changes are listed in Table 2 Changes were detected in all expts except one The first changes usually S-T segment depression appeared after 1–2 min and often persisted throughout the observation time The periods of supraventricular extrasystoles were of short duration and of no hemodynamic importance

Peripheral blood flow changes

The control values for the organ blood flows w in the same range as has been previously dem strated in monkeys (Forsyth et al 1968) Follo challenge there was a decrease in the blood flo all organs studied (Table 3) The mean decr was 97 % in the kidneys 91 % in the pancreas 99 % in the spleen The mean decrease in the art al blood flow of the liver was 44 % and of t abdominal fat 77 % The muscle blood flows d creased by 50–60 % The total myocardial bl flow decreased by 37 % The blood flow of the cerebral hemispheres was equally distributed b in the control situation and during shock. Dur shock there was a mean decrease of 44 %

Myocardial performance

The values for myocardial performance are sented in Table 4 The total myocardial blood in per cent of CO increased from a mean of 4 8 2 % The coronary vascular resistance did change significantly The blood flow of the l ventricular free wall sho consistent ch

1 *Mean alues (±S.E.) for cardiac output (CO) heart rate (HR) stroke volume (SV) total eral resistance (TPR) and pulmonary vascular resistance (PVR) at the control measurements and 4 ter challenge ith antigen (n=8 except for PVR here n=6)*

values were calculated by paired -tests. For PVR, however the sign test was used for calculating the *P* value, of the extremely uneven distribution during shock

	Control	Shock	Mean change from own control (%)	*P*
l min kg⁻¹)	218±25	52±11	−75	<0.01
ats min)	198±8	235±8	+19	<0.01
l)	3 1±0.4	0.6±0.1	−79	<0.01
mmHg min ml⁻¹)	0.193±0.027	0.426±0 039	+140	<0.01
mmHg min ml⁻)	0.024±0 004	0.[illegible]2±0.119	+984	0.03

the blood flow of the left entricular free wall ased by 50%. The available oxygen for the entricular myocardium did not alter signifi- y whereas for the left it decreased by a mean 1%. The right and left ventricular stroke work ased by 49% and 81% respectively. The r lated control blood flow ratio (Table 5) for LV t endo/epi was 1 11 and for LV apex endo/epi and the corresponding values during shock 0 84 and 0.87 respectively. The control ratio V/RV was 1.56 and a decrease to 0 83 was d during shock.

USSION

animals used in this study had high titers of umin-specific hemagglutinating antibodies of ominantly the IgG class (Smedegård et al. . The reaction thu elicited by ovalbumin in- ns may be classified as aggregate anaphylaxis

(Becker & Austen 1976). Upon challenge the monkeys responded with a severe state of shock with a high pulmonary vascular resistance a low cardiac output and profound hypotension. A similar reac tion has been reported by Pavek (1977). It has been shown by Churchill & Cope (1929) in cats that as a result of a reflex mechanism high pressures in the pulmonary circulation may cause bradycardia, a fall in blood pressure and inhibition followed by stimulation of the respiration. Since an increased pulmonary arterial pressure was prominent ery early in the present experiments this reflex may be responsible for the initial transient period of bradycardia and the initial fall in systemic arterial pressure. A peripheral vasodilatation which is also considered to be a part of this reflex (Aviado et al 1951) may explain the initial fall in the right atrial pressure. The findings on measurement of the re spiratory mechanics in these animals (Revenäs et al 1979) support the idea of involvement of this reflex since challenge with ovalbumin resulted in suppression of the respiration followed by stimulation.

The stroke work decreased after challenge. This decrease was smaller for the right ventricle than for the left owing to an increased pressure load on the right ventricle. Furthermore, the right ventricular stroke work was reduced despite a high filling pressure whereas the reduction in left ventricular stroke work coincided with a low filling pressure indicating that the mechanisms underlying the low output state on the right and left side were different. This suggests that right ventricular failure occurred early in the reaction. The finding at autopsy that the right entricle was distended

e – *Electrocardiographic (ECG) changes in 8 ey) following hallenge th ovalbumin*

he 3 animals hich had periods of supraventricular ystoles, 2 also had S-T changes and one had T-wave rsion

en of ECG change	No of animals
detectable change	1
raventricular (extras) ystoles	3
tricular (extras) ystoles	0
vation and/or depression the S-T segment	6
ave inversion	2

Table 3 *Mean values (± S E) for regional blood flows at control measurements and 4 min after cha[illegible]*
The *P* values were calculated by paired *t* tests

	n	Control (ml min 100 g)	Shock (ml min 100 g)	Mean change from own control (%)	*P*
Kidney left	8	578±62	15±4	−97	[illegible]
Kidney right	8	644±79	19±5	−97	[illegible]
Pancreas	8	11±38	18±9	−91	[illegible]
Spleen	8	287±35	2±1	−99	[illegible]
Liver (hepatic artery)	8	4 ±11	1±5	−44	[illegible]
Abdominal fat	8	4±5	4±1	−72	[illegible]
Pectoralis major left	4	7 5±3.3	2 4±0 8	−54	[illegible]
Pectoralis major right	8	6.8±1	5±0 6	−59	[illegible]
Biceps left	8	6 0±1	2.0±0.4	−57	[illegible]
Biceps right	8	4 6±1.3	1 6±0 4	−55	[illegible]
Heart (coronary circulation)	8	52± 8	147±36	−37	[illegible]
Cerebral hemisphere left	6	102±16	67± 5	−44	[illegible]
Cerebral hemisphere right	6	106±1	65±2	−44	[illegible]

(Smedegård et al 1979) supports the assumption of right heart failure The two animals which died during the observation period had the largest decrease in left atrial pressure and cardiac output and the largest increase in pulmonary vascular resistance This suggests that the reaction was more severe but the general pattern was the same as in the other animals In addition however the left atrial pressure increased in the last 5 to 10 min before death This suggests that left ventricular failure was a terminal consequence of the severe shock

The increased pulmonary arterial pressure and the low cardiac output were evidence of the increased pulmonary vascular resistance It is most likely that venous constriction contributed [illegible] cantly to the overall increase in resistance [illegible] pulmonary circulation since the lung [illegible] heavier (Smedegård et al 1979) in the absenc[illegible] high left atrial pressures The increased lung [illegible] was due to pooling of blood and formation of [illegible] stitial oedema (Smedegård et al 1979). Pooling [illegible] blood also occurred within the right heart and [illegible] veins resulting from the right heart failure, as [illegible] cated by high central venous pressures. The po[illegible] was probably extended to the periphery All [illegible] events resulted in a decreased circulating [illegible] volume and a relative hypovolemia.

After challenge the blood flow decreased [illegible]

Table 4 *Mean values (± S E) for coronary vascular resistance (CVR) and myocardial blood flows, [illegible] work minute work and available oxygen for the right (RV) and left ventricle (LV) (n=5) at co[illegible] measurements and 4 min after challenge*
The *P* values were calculated by paired *t*-tests

	Control	Shock	Mean change from own control (%)	*P*
Myocardial blood flow (ml min 100 g)	277±35	174±51	−38	[illegible]
Myocardial blood flow (% of cardiac output)	4 3±0.5	8 2±1 6	+87	[illegible]
CVR (mmHg min ml 100 g)	0 41±0 06	0.52±0 11	+ 7	[illegible]
RV blood flow (ml min 100 g)	88±41	224±70	+7	[illegible]
LV blood flow (ml min 100 g)	348±52	182±59	−50	[illegible]
RV stroke work (mJ)	8 0±1 6	3 4±0 4	−49	[illegible]
LV stroke work (mJ)	48 5±8 4	8 5±1.3	−81	[illegible]
RV minute work (J)	1.5±0 3	0 8±0.1	−38	[illegible]
LV minute work (J)	9 1±1 3	1 9±0 3	−76	[illegible]
RV available oxygen (ml O_2 min 100 g)	9 9±6 8	25 6±8 7	−9	[illegible]
LV available oxygen (ml O_2 min 100 g)	45 6±9 1	20 9±7 6	−57	[illegible]

3 Mean values (±S E.) for myocardial blood flow ratios at the control measurements and 4 min challenge (n=6)
P values were calculated by paired t-tests

	Control	Shock	Mean change from control	P
ventricular base, endo/epi ratio	1 11±0.05	0 84±0.05	−0.27	0.04
ventricular apex, endo/epi ratio	0 92±0 07	0 87±0.13	−0.06	0.64
right ventricular ratio	1.56±0 14	0.83±0.09	−0.74	<0.01

s studied, but to different degrees in different s. The decrease was most marked in the those organs (kidneys, pancreas and spleen) maller in the muscles, where it paralleled the se in mean arterial pressure. The smallest tion in blood flow was found in the brain, ardium and liver The redistribution of the to tal organs at the expense of flows in other ns conforms with earlier data from monkeys cted to hemorrhagic or endotoxin shock yth et al 1970 Rutherford et al. 1976) How- the magnitude of the blood flow changes in the nt study was greater even though the degree potension was similar which is probably ex ed by a greater cardiac output reduction.

ere was no decrease in coronary vascular re- nce, which might otherwise be expected when perfusion pressure falls as is seen in hem- gic shock (Ledingham 1976 Carlson et al. an Achern 1976) This might have been to a release of anaphylactic mediators (Levi et 976, Aabut et al. 1978) causing vasoconstric and interfering with the mechanisms regulating blood flow in the myocardium In the present ly an appropriate redistribution of the blood from the left to the right myocardium was rved, and thus a marked effect of vasoconstric anaphylactic mediators was unlikely An native explanation for the unchanged re nce may be that the myocardium was suffi- ntly perfused The amount of oxygen available myocardial consumption is related to the coro- y blood flow since there is normally small erve for increased extraction (Katz & Feinberg 8). However a decrease in blood flow does not itself imply myocardial ischemia, as the work of heart normally decreases in shock (Opdyke & rman 1947). In the present study both the heart rk and myocardial blood flow were reduced. wever the blood flow was redistributed from the left ventricular myocardium to the right, probably as a consequence of the relative change in he modynamic load on the two ventricles. This has been shown to occur in pulmonary embolism (Oldham et al. 1974 Sestier et al. 1978) This redistribution resulted in an unchanged supply of oxygen to the right ventricle A comparison between the magnitude of the change in minute work and the oxygen supply per minute for the left and right ventricle, respectively showed that the decrease in work was greater than the decrease in oxygen supply Provided the utilization of oxygen was not impaired this may indicate that the oxygen supply was sufficient. However since the minute work is not linearly related to the oxygen demand, the present data cannot entirely rule out myocardial oxygen deficiency This relative increase in oxygen supply does not eliminate the possibility that regional myocardial ischemia was present. The relevance of LV endo/epi ratios in determining the presence of subendocardial ischemia has previously been discussed by Brazier et al (1974) among others. Carlson et al. (1976) found a decrease in the LV endo/epi ratio in dogs during hemorrhagic shock. This has recently been confirmed by Adiseshiah & Baird (1978) who suggested that an important contributory factor to the decreased ratio was a decreased left ventricular preload, resulting in decreased transmural gradient in the diastolic myocardial tissue pressure The reduction of the tissue pressure was greater in the epicardial than in the endocardial layer thus favouring the epicardial blood flow Utley et al (1974) predicted from a mathematical model that a decrease in the volume of the left ventricle may cause a distortion of the subendocardial vessels and consequently an increased resistance in that layer of the muscle In the present study a reduced preload and changes in the transmural pressure gradient could very well have explained the observed decrease in the LV endo/epi

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Kidney right	8	644±79	19±5	−97	<[illegible]
Pancreas	8	11±38	18±9	−91	<[illegible]
Spleen	8	_87±35	±1	−99	<[illegible]
Liver (hepatic artery)	8	4_±11	1±5	−44	[illegible]
Abdominal fat	8	?4±5	4±1	−72	[illegible]
Pectoralis major left	4	7.5±3 3	2.4±0 8	−54	[illegible]
Pectoralis major right	8	6 8±1	2.5±0 6	−59	[illegible]
Biceps left	8	6.0±1._	2.0±0 4	−57	[illegible]
Biceps right	8	4 6±1.3	1 6±0 4	−55	[illegible]
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RV minute work (J)	1 5±0 3	0 8±0 1	−38	[illegible]
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RV available oxygen (ml O min 100 g)	?9 9±6 8	_5 6±8.7	−9	[illegible]
LV available oxygen (ml O_2 min 100 g)	45 6±9 1	20 9±7 6	−57	<[illegible]

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...cipal mechanisms controlling penile retraction and ...trusion in rabbits

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The effects on penile volume of nerve stimulations and drugs injected into the systemic circulation were studied plethysmographically. Dilator responses at selective perfusion of the penile artery were studied by measuring the perfusion pressure. The main results and conclusions are: The penis has an adrenergic vasoconstrictor supply coming from the sacrococcygeal parts of the sympathetic chains. A very low (0.2 Hz) vasomotor tone keeps the penis relaxed. If this tone is interrupted the penis will protrude but autoregulation will soon take over and eventually produce hyperinvolution of the penis. Two vasodilator paths, both with pelvic ganglionic relays were found. 1) The pelvic parasympathetic nerves, probably having mainly non-cholinergic postganglionic neurons and operating quite effectively at low frequencies. 2) The sympathetic hypogastric nerves, presumably having at least partly cholinergic postganglionic neurons which, apart from muscarinic dilation of minute inflow resistance vessels to the erectile tissue, may also work by suppression of excitatory adrenergic neurotransmission. The pelvic and hypogastric vasodilator outflows work synergistically. The vasoconstrictor nerves are very strong and efficient antagonists of the vasodilator nerves.

Key words: Penis, erection, autonomic innervation, vasodilation, smooth muscle

...rimary event in mammalian penile erection is ...ation of the arterial branches forming the inflow ...istance vessels to the cavernous bodies. This ...ds to situation where the outflow resistance ...ceeds the inflow resistance and consequently the ...ectile tissue will be engorged. Conversely vaso-...striction forms the basis of the flaccid state of ...e penis.

The nature of penile vasodilation has been a ...atter of dispute for long time (vide Klinge & ...östrand 1974). Two main issues have been de-...ated. 1) Are the pelvic parasympathetic nerves, ... the nervi erigentes of Eckhard (1863), the only ...ath for penile vasodilator fibres? This seems to be ...e predominant view apparently based on the ...orks of Langley & Anderson (1895) and Semans & ...angworthy (1938) where other routes are refuted. However there are continental reports indicating ...at erector fibres also could travel along the sym-...athetic system, especially the hypogastric nerves (Eckhard 1876, François-Franck 1895 Bacq 1935 Bessou & Laporte 1961 Thelen, Renders & Rectem 1969). 2) Are the dilator fibres cholinergic, as generally assumed or not? In fact, this question is much older than the concept of cholinergic nerves and goes back to a dispute on whether atropine paralyzes (Nikolsky 1879) the nervi erigentes or not (Anrep & Cybulski 1884). In this century a cholinergic transmission of erection has been proposed by e.g. Henderson & Roepke (1933) Bacq (1935) Oppenheimer (1938) Bessou & Laporte (1961) and Hukovic & Bubic (1967) while it has been doubted by e.g. Luduena & Grigas (1966) and Dorr & Brody (1967).

We have criticized the pharmacological evidence presented by earlier investigators the main objections being the combined use of preganglionic stimulation and very high frequencies which make it difficult to evaluate the point of attack and the effect of drugs such as atropine and physostigmine (Klinge & Sjöstrand 1974). Concerning the putative sympathetic erector fibres there is also an objection

ratio Moreover since tachycardia occurred this would probably have augmented the decrease (Domenech & Goich 1976) The pathophysiological significance of the relative decrease in subendocardial flow in the present study is unknown but since there were no signs of left ventricular failure early in the reaction this may be of minor importance

It can be concluded from the present observations in monkeys subjected to aggregate anaphylax is that in these animals challenge with ovalbumin results in severe circulatory shock with a markedly reduced cardiac output and profound hypotension The most important hemodynamic alteration is an increased resistance in the pulmonary circulation resulting in acute right heart failure and a central pooling of blood

The technical assistance of Kerstin Lövgren and Ove Forsberg is much appreciated

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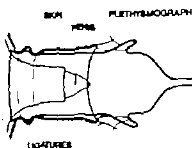
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Schematic dra ing of the glass plethysmograph in rontal ew The plethysmograph is connected to ssure transducer to Grass polygraph The degree ension of the rabbit penis, which is retracted in the illustration, also directly nable. For further ex ions see Material and Methods

the level of the thyroid cartilage. The cut nerves placed on book-shaped platinum electrodes (? mm shielded underneath ith plexiglass and provided upper side ith movable plexiglas rod for fixing ve. All electrodes were provided with holders de- specially for each nerv The holders were fixed to peration table in order to keep the electrodes in on during the expt Paraffin oil in ample amount was ar isolation. The nerves ere stimulated ith square pulses (4–6 ms) and supramaximal" oltage (15–25 Unless otherwise stated, all stimulations were bilat- The usual stimulation period was 20 s. This will be ed to as standard stimulation In some frequency-re e expts we stimulated until the response became ized at fixed level This will be referred to as lation th unlimited train.

Section of lumbosacral sympathetic fibres prior to
Six rabbits ere pretreated with promethazine (16 kg in) and anesthetized with sodium pentobarbital 200 µmol/kg) The abdomen was opened along nea alba and the paravertebral chains were dissected around the ganglia at L_4 The cranial and caudal ections of these ganglia and those at L_6 and S_1 were ved, hereafter the abdomen was closed. The ani- ere used for xpts 3 4 6 14 or 21 d afte ation.

al considerations in the anal sis of the effects muscarinic drugs on erectile esponse

se of antimuscarinic drugs as atropine or scopola- n in re expts. on rabbits is encumbered with many ks. Many rabbits have an inherited atropinester plasma, liver and several other organs, which rapid- activates both atropine and scopolamine (for ref see kalow 1962, Cauthen et al 1976) There further is the lem of reaching sufficient concentration of drugs in nally poorly perfused organs as the penis. Therefore effects of atropine and scopolamine on the response of penis to pelvic or hypogastric nerv stimulation were studied against the effect of the drugs on the response of heart to stimulation on the right agus nerv (20 s, 25 Hz). This response is one of the most atropine sensitive cholinergic nerve effects (g. Ursillo 1961). Consequently only in xpts. where this effect could be blocked one might obtain significant pharmacological data on the possible muscarinic nature of the erectile response.

W could divide our rabbits into 3 groups. A small group (only 3 rabbits) in which the agal bradycardia could be blocked for several h by moderate doses (4–20 µmol/kg) of the drugs, main group in which just short-lived (3–15 min) suppression of the bradycardia was obtained with even huge doses (70–170 µmol/kg) of the alkaloids, and finally one group where even grotesque doses (more than 16 µmol/kg) were without any effect. These groups were defined as atropine sensitive, semiresistant and resistant, respectively The difficulties in evaluating the effects of the antimuscarinic drugs prompted us to do the perfusion expts Atropine and scopolamine also ha e ganglionic blocking properties (vide Klinge & Sjöstrand 1974) which could impair the analysis. This possibility was checked by recording contractions of the seminal esicle to injected noradrenaline and to hypogastric nerve stimulation hich is preganglionic to that organ (vide Sjöstrand 1965). We obtained no clear-cut evidence for ganglionic action of the alkaloids hen doses of 4–40 µmol/kg were used. However with the higher doses which we had to use in the perfusion expts and also in the expts. on atropine sensitiv rabbits, an interference of ganglionic or post-ganglionic nicotinic point of attack cannot be excluded.

Equipment

Recordings ere made on Grass polygraph. Statham transducers were used for pressure recordings and a Grass olumetric low-pressure transducer (PT5) was used in plethysmography A Sigma motor pump was used in the perfusion expts. Gras S4, S44, S5 or SD 9 stimulators delivered the shocks. Slow infusions were made with a Braun infusion pump Temperatures were measured ith Gold Brand precision thermometers and a Harvard respirator as used for artificial ventilation

Some ritical omments on the methodology

During evisceration the rectal temperature fell to 35–33°C and stayed at this level throughout the expt. despite warming. Together with the damping of the recording system this means that our quantitative data are sluggish compared to those of real life The airfilled ystem in plethysmography had of course the errors of this method. Nervous injuries due to preparation should not be over looked Especially the pelvic nerves were difficult to prepare satisfactorily in contrast to the easy hypogastric nerves

Drugs

The following pure salts, bases and peptides were used. *l*-noradrenaline-*d*-bitartrate, *l*-adrenaline-*d*-bitartrate, terbutaline sulphate, phenoxybenzamine hydrochloride phentolamine methane sulphonate, propranolol hydrochloride, guanethidine sulphate, acetylcholine chloride, neostigmine bromide, atropine sulphate, scopolamine bromide decamethonium bromide mecamylamine hydrochloride, hexamethonium bromide, histamine chloride, mepyramine maleate, promethazine chloride, cimetidine methysergide dimaleate, arginine vasopressin, oxytocin,

against the use of α-adrenergic blocking ergot compounds (Bacq 1935 Bessou & Laporte 1961) in order to make the vasodilation clear because the observed effect could be due to adrenergic β receptors

Experiments on various isolated smooth muscle effectors of penile erection have led us to the conclusion that neurogenic relaxation of these smooth muscles is hardly cholinergic although cholinergic nerves may contribute to erection by prejunctional inhibition of excitatory adrenergic neurotransmission to these effectors (Klinge & Sjöstrand 1974 1977*a* and *b*) In this report we present results from in vivo studies performed with the aim to obtain information on the basic myogenic and neurogenic control mechanisms of penile volume The rabbit having a penis rather similar to that of man (Fujimoto & Takeshige 1975) was chosen as experimental animal A preliminary account of the results has been presented (Sjöstrand & Klinge 1978)

MATERIAL AND METHODS

Animals

This and the subsequent report (Sjöstrand & Klinge 1979) are based on expts on 58 rabbits of different breeds 3 were albinos of the New Zealand race or native strains B wt was in the range of [illegible]0–5.5 kg.

Operative procedures

1 *General preparation and care* The rabbits were starved for 12–18 h and anesthetized with urethan (16–20 mmol/kg i v) They were tracheostomized and a T-tube was inserted The abdomen was opened with a midline incision The inferior and superior mesenteric arteries and the corresponding branches of the portal vein were tied and the gut was removed between the duodenum and the oral end of the rectum The ureters were cannulated and the bladder was emptied The vessels of the bladder were tied and the bladder was resected above the trigonum area. A cotton wool pellet soaked in vaseline was introduced into the urethra through the opened bladder and placed at the level of the pubic bone in order to prevent secretion from the male sex accessories into the penis The vasa deferentia were ligated in the ampullary region and the seminal vesicle was slit in its tips and the secretion was removed A catheter for i v infusions was introduced into either a femoral or an external jugular vein In some expts a catheter for i a injections was introduced into a femoral or a subclavian artery and pushed to the aortic origin of the vessel Arterial blood pressure was recorded from one of the carotid arteries (usually the left) or femoral artery In expts. in which the sympathetic chains were cut and stimulated the external iliac vessels were usually tied in order to prevent drop of blood pressure and pooling of blood in the hind legs In some expts. artificial ventilation was given under decamethonium ([illegible] μmol/kg i v) blockade

After evisceration the animals received [illegible] ml 6% dextran in saline (Macrodex® Pharmacia) per kg b wt During the course of the expt. the rabbits received [illegible] ml each of the following solutions per h. glucose [illegible] (both 0.56 M) $NaHCO_3$ 1 6×10 [illegible] M, 6 [illegible] dextran [illegible] Ringer or saline

2 *Plethysmography of the penis* The attachment of the penile skin to the rectum was severed. The opening of the penile skin as well as that in the rectal skin were [illegible] closed by sutures The preputium was elevated and its inner layer was separated from the penis by a [illegible] incision through the attachment beneath the glans [illegible] Via this incision the entire penis was stripped by [illegible] dissection The skin tube covering the penis was left in continuity with the pubic and the perineal skin The skinned penis was lubricated with paraffin oil and [illegible] greased glass plethysmograph provided with 3 hooks [illegible] introduced into the skin tube and placed around the [illegible]tulum The plethysmograph was resting on the [illegible] formed by the perineal striated muscles encasing the crura and the bulb of the penis. Ligatures from the [illegible] skin to the hooks kept the plethysmograph in place [illegible] complete sealing was achieved by tying threads [illegible] the base of the penile skin and plethysmograph (Fig [illegible] The recording system was filled with 7–8 ml of [illegible] care was taken to keep the room temperature constant.

3 *Selective perfusion of the penile artery* The [illegible] the pubic perineal and sacrococcygeal regions and [illegible] the hips were flayed Via blunt dissection in the [illegible] femoral groove cutting off covering muscles and [illegible] ing the tuber ossis ischii with its muscular insertions the internal pudic artery was identified in Alcock's canal [illegible] the 5 New Zealand rabbits used in this type of expt there was quite a difference in size of the penile arteries on the two sides The smaller artery was ligated while the larger was cannulated at its origin i e where the terminal pudic artery divides into the penile and rectal branches [illegible] visible penile veins were ligated centrally and cut peripherally and the tip of the glans penis was cut off to permit outflow of perfusion fluid The penile artery was perfused with Tyrode solution (Klinge & Sjöstrand 1974) which contained [illegible]% Ficoll® (Pharmacia), M w about 7[illegible] and 1.5% Ficoll® M w about 47000 as colloid and was aerated with 6.5% CO[illegible] in O_2 and kept at about 3[illegible]°C Perfusion rate was 1 75–3 0 ml/min and histamine ([illegible] 5 0×10$^{-[illegible]}$ M) was added to induce sufficient [illegible] i e an effective initial perfusion pressure close to [illegible] mmHg The pressure was measured via a T-tube inserted into the arterial cannula. An inlet for injections was placed between the reservoir and the pump close to the latter

4 *Dissection and stimulation of nerves* Good descriptions of the pelvic hypogastric and pudic nerves in the rabbit and of the approach to them are given by Langley & Anderson (1895 1896). These guidelines were essentially followed The lumbosacral sympathetic chains were identified and prepared at the level of L_6–S_1 The left splanchnic nerve was isolated rather close to the adrenal In three expts in which adrenomedullary stimulation was performed the right adrenal was separated from the circulation by tight ligatures The vagus nerves were isolated [illegible]

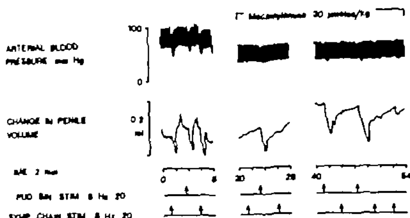


Fig. 3. Rabbit, 3.1 kg, urethan, external iliac vessels tied, lateral position. Sympathetic chains cut at L_6–L_6, electrodes at L_7 and on left pudic nerve. Alternate stimulations. Left panel: Control: both stimulations reduce penile volume. Note "rebound" increase in volume after stimulations. Middle panel: 10 min after mecamylamine infusion. The response to stimulation of the sympathetic chains is blocked but not that to pudic nerve stimulation. Right panel: 30 min after ganglionic blockade. Slight reappearance of the effect of chain stimulation is noted in the last two stimulations. Pudic nerve stimulations give marked responses. The volume of the penis gradually increased which may partly have been due to accumulation of vasodilating metabolites into the blood as consequence of the low pressure.

…/kg) and hexamethonium (28 µmol/kg) pro… clear-cut penile protrusion concomitantly … a lowering of the blood pressure (Fig. 4). Simi… protrusions were produced by local infiltration …docaine (7.4×10^{-2} M) on the lumbar sympathet… …ains or the pudic nerves (Fig. 4). Penile retrac… …produced by vasoconstrictor stimulation (0.05–16 Hz) was blocked by guanethidine (6.5–13 µmol/kg), dihydroergotamine (0.5–2.0 µmol/kg), phenoxybenzamine (1.5 µmol/kg) or phentolamine (2.5–14 µmol/kg). The latter two drugs immediately produced marked drop in blood pressure and clear cut penile protrusion in rabbits with intact constrictor pathways (cf. Fig. 2). Under these circumstan-

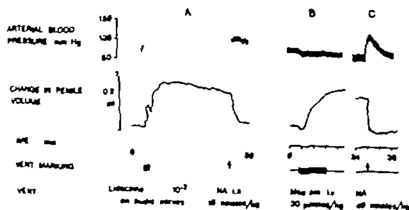


Fig. 4. A. Rabbit, …1 kg, urethan, ventral position. Infiltration of lidocaine around both pudic nerves along their dorsal course produces protrusion of the penis. Intra-arterial injection of noradrenaline leads to prompt retraction of the penis. *B.* Rabbit, 4.0 kg, urethan, dorsal position. Mecamylamine causes pressure drop and penile tumescence. *C.* In the same rabbit noradrenaline instantly causes detumescence although it considerably raises the blood pressure.

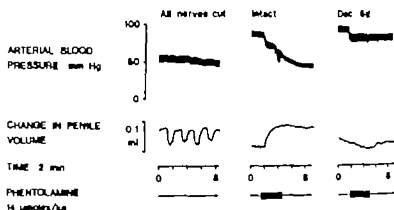


Fig 2 Spontaneous changes in and effect of phentolamine on penile volume. Blood pressure in this and the other figures is recorded from the left carotid artery Left panel Rabbit 3 0 kg urethan. Rhythmic changes in penile volume 2 h after severing of the sympathetic chains the pelvic and hypogastric nerves Middle panel Rabbit, 4 kg, urethan Phentolamine decreases blood pressure and increases penile volume in the "intact" rabbit Right panel Rabbit 4 1 kg urethan. Sympathetic paravertebral decentralization of the penis 6 days before expt The pressure drop after phentolamine is moderate. Penile volume is not increased but rather decreased

isoleucine⁵-angiotensin, lidocaine hydrochloride sodium pentobarbital and urethan For manufacturers see Klinge & Sjostrand 1974 1977*a* and *b* Dihydroergotamine mesylate was used in the form of the pharmaceutical product Orstanorm® (Sandoz).

RESULTS AND COMMENTS

Evidence for myogenic tone and automaticity in penile vascular bed (Fig. 2 See also Figs 3 6 and 7)

In vitro studies of various smooth muscle effectors of penile erection indicate a high degree of automaticity in most of them (Klinge & Sjöstrand 1977*a*) This seemed to be the case also in vivo Rhythmic oscillations in penile volume having similar periodicity as rhythmic contractions in isolated cavernous bodies of rabbits were now and then observed in about half of the animals (Fig. ?) These oscillations were not eliminated by section of any of the penile nerves They could be seen for some minutes after circulatory arrest in the animal Thus they might be due to synchronic contractions and relaxations of many smooth muscle fibres in the cavernous bodies rather than to periodicity in the inflow and/or outflow resistance vessels However since removal of the vasoconstrictor tone never resulted in full penile protrusion (see below) also the inflow resistance vessels must normally have myogenic tone

Vasoconstrictor innervation of excitatory and penile smooth muscles (Figs ?–6 and Table I)

A *Anatomy* According to among others L... (1866) François-Franck (1895) and Langley & A...derson (1895) the main part of the vasoconstrictor fibres to the penis come from the lumbosacral sympathetic chains and reach the penis via the p... nerves This was confirmed Since stimulation of the pudic nerves rarely produced as forceful responses as stimulation of the sympathetic ch... (L_4–S_1) and since severing of the chain produced greater protrusion than cutting of the pudic nerves close to the penis many fibres must join the p... artery at quite a distance from the penis. Ex... investigators (for ref see Klinge & Sjöstrand ... have found some vasoconstrictor fibres in the hypogastric and pelvic nerves In ? expts. only ... faint retractions preceding the usual protrusion elicited by stimulation of these nerves. Therefore these routes seem to be exceptional for constrictor fibres in the rabbit

B *Pharmacology* Mecamylamine (30 µmol/... suppressed the response to stimulation of the sympathetic chains (L_4–S) but not that to stimulation of the pudic nerves (Fig 3) I e the synaptic relays of the constrictor pathway seem to be located ... in the sacrococcygeal part of the chain (cf Langley & Anderson 1895 1896) In rabbits with ... ered vasoconstrictor nerves mecamylamine (1...

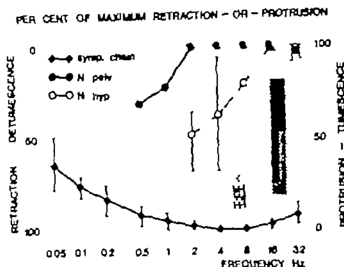


Fig. 4. Frequency-response relationship between penile volume and stimulation with "tailored" pulse train of vasoconstrictor or dilator nerves. 100% Protrusion = 0% retraction is the maximum volume obtained by stimulation of vasodilator nerves. 100% Retraction = 0% protrusion is the minimum volume obtained by stimulation of vasoconstrictor nerves. All rabbits used for these plots had mean arterial pressure between 85–115 mmHg. Frequency on logarithmic scale. Each point represents mean and range of 3 expts. Dotted area represents range of resting volume calculated from 10 expts. The area with most dots indicates the most stationary level. Resting level corresponds to vasoconstrictor tone in the range of 0.1–0.5 Hz. Striped area indicates volume after section of sympathetic chains. Darker part indicates the initial protrusion, lighter one shows the level after retraction. Calculated from 10 expts. Note the range. For further explanations see text.

s calculated from the plethysmograms re presented in Table I. The average rate to the peak (as well as the rate during the initial rapid phase) of volume decrease are given. The rate of the initial phase reaches maximum at about 4 Hz while average rate of volume decrease continues to rise up to 8–16 Hz. This would fit with the above mentioned interpretation of the shape of the curve to vasoconstrictor stimulation because vasoconstrictor responses of capacitance vessels have maxima at lower frequencies than those of resistance vessels (cf. Mellander 1960, Mellander & Johansson 1968). In this context it should be stressed that the main volume decrease occurred during the initial rapid phase (Fig. 3).

2. Estimation of resting vasomotor tone to the penis. In Fig. 6 the results of 10 expts in which the range of the resting level was calculated against the maximum retraction obtained by vasoconstrictor stimulation and the maximum protrusion obtained by vasodilator stimulation are summarized (dotted area). The dotted area corresponds to vasoconstrictor stimulation in the frequency range of 0.05–0.5 Hz. The most stationary level (most heavily dotted area) in each expt. corresponds to 0.1–0.2 Hz. That the "normal" vasoconstrictor tone in fact was within this range could be verified in expts where after protrusion due to section of the sympathetic chains the precutting level of penile volume was restored by stimulation with 0.1–0.3 Hz. Thus, provided that there normally is a fairly uniform activation of the penile vasoconstrictor fibres their vasomotor tone would be of the order of 0.2 Hz. This vasomotor tone is lower than that calculated in similar way for most other vascular beds in anesthetized animals (for ref. see Mellander & Johansson 1968, Folkow & Neil 1971, Öberg 1976).

Influence of the adrenal medulla and effect of injected noradrenaline and adrenaline on penile volume (Fig. 4)

A. Adrenal medulla. Cutting or infiltration with lidocaine of splanchnic nerves had no effect on

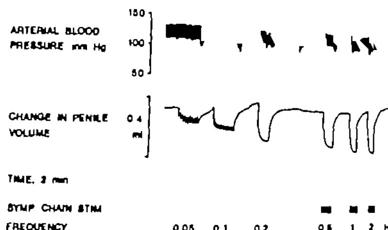


Fig 5 Rabbit 3.8 kg, urethan Sympathetic chains cut at L_6–L_7 electrodes on L_7–S_1 external iliac vessels tied Responses to stimulation of the chain with varying frequencies Note that the effect of individual pulses can be traced in the plethysmogram at 0 05 and 0 1 Hz Note also biphasic volume decrease during vasoconstrictor stimulation i.e. and initial rapid phase and a slower second phase The protrusion after section of the chains was unusually great in this expt

ces 6 5–13 μmol/kg of guanethidine produced only a drop in blood pressure without protrusion of the penis whereas a transient protrusion was seen after 65 μmol/kg. Dihydroergotamine had but little effect on blood pressure and penile volume but if the sympathetic chains were cut and the penis protruded this compound retracted the penis

C *Effect of section of vasoconstrictor pathways* As mentioned above also mechanical interruption of the vasoconstrictor tone produced increase in penile volume The usual phenomenon was a rapid protrusion followed by a slow (0 5–4 h) or sometimes rapid retraction In Fig 6 the initial as well as the final volume range of 10 expts is illustrated (striped area) As seen the initial protrusion varied from just slightly above the resting level of the intact penis to about 80% of maximum protrusion produced by stimulation of vasodilator nerves In most expts the protrusion produced by cutting the vasoconstrictor pathways was 40–60% and the following spontaneous retraction reduced the volume to a level corresponding to the upper limits of the resting volume of the intact penis i e to 20–30%

D *Response to stimulation of the sympathetic chains shape and frequency response relationship* Penes protruded after section of the sympathetic chains responded even to single pulses with a slight retraction and on continuous low frequency stimulation the responses to individual shocks could be traced (Fig 5) As seen in this figure the response to vasoconstrictor stimulation had two phases an initial rapid decrease in volume and an additional second slow decrease This was especially within the frequency range of 1–4 Hz. Volume decreases in two phases with different slopes have been described on vasoconstrictor stimulation of other organs The rapid initial phase has been interpreted as due to expulsion of blood from capacitance vessels while the subsequent slow phase has been ascribed to absorption of extravascular fluid caused by relatively more pronounced vasoconstriction in pre than postcapillary resistance vessels (vide Mellander 1960). Accordingly we consider it likely that the initial rapid phase may chiefly be due to squeezing out of blood from the penis as a consequence of contraction of the smooth muscles in the walls and the trabeculae of erectile tissue which could be regarded as a kind of capacitance section in the penile bed. The later slower phase on the other hand, may mainly be due to emptying of the erectile tissue because of greater resistance in the inflow than in the outflow vessels

In Fig. 6 the frequency response relationship between penile volume and unlimited train stimulation of the sympathetic chains is presented The points are based on animals in which cutting of the chains produced 60–80% initial protrusion Maximum retraction is obtained with 1–4 Hz. While the plot in Fig 6 just gives a "static" conception of the effect of vasoconstrictor stimulation the more "dynamic" values of the corresponding emptying rates of the

	16	32
	1.25±0.25	0.76±0.15
	1.15±0.30	1.05±0.58
	1.05±0.76	0.67±0.41
	4.06±0.93	2.19±0.73
	2.67±1.40	2.09±1.21
	1.72±1.65	1.60±0.80

…that there was no significant tonic discharge …e dilator fibres.

Pelvic pathway 1) *Pharmacology* The dilator …onse was blocked by mecamylamine, the doses …g in general higher than those required to block vasomotor tone i.e. 30–60 μmol/kg. This effect … best seen in animals in which the sympathetic … had been cut prior to the expt. in order to …imize the decrease of blood pressure (Fig. 7) … our stimulation was preganglionic (cf. Langley & Anderson 1895). We did not observe any overt effect of even very large doses of atropine (140 μmol/kg) or scopolamine (250 μmol/kg) in atropine sensitive or semiresistant rabbits on the erectile response to pelvic nerve stimulation (20 s 0.5–16 Hz). This also applies to propranolol (10 μmol/kg) mepyramine (.5 μmol/kg), cimetidine (40 μmol/kg) and methysergide (4.3 μmol/kg). Furthermore the dilator response of the selectively perfused penile artery to pelvic nerve stimulation (6–12 Hz, 30 s 2 expts.) was not overtly affected even by long time perfusion with ~6×10⁻⁶ M scopolamine (Fig. 10*c*). This concentration of scopolamine blocked the effect of acetylcholine injected into the penile artery (Fig. 10*b*).

2) *Frequency-response relationship* Threshold frequency for a response was 0.1–0.5 Hz. With 0.5 Hz steady and reproducible effects were obtained in most expts. Fig. 6 shows a frequency-response plot to unlimited stimulation. The expts. were performed on rabbits with intact vasomotor tone. Maximum protrusion is obtained within 1–… Hz. While it is of interest for the understanding of long lasting erections as may be the case e.g. in man, the curve gives however but little of the dynamics of erection. The corresponding filling rates are therefore presented in Table 1 in which the calculated average as well as maximum rates are given. The erectile responses were not distinctly biphasic as were the retractile responses. In general there was a lag phase, a short slow phase and a rapid phase which eventually levelled off. At increasing fre-

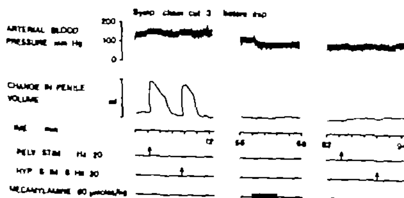


Fig. 7. Rabbit, 4.1 kg, urethane, sympathetic chains cut 3 days prior to expt. Left panel: Responses to pelvic and hypogastric nerve stimulation. Note size and duration of responses and also rapid phase of retraction. Middle panel: Mecamylamine is without significant effect on penile volume (cf. Fig. 4B). Right panel: After ganglionic blockade the responses to hypogastric and pelvic nerve stimulation are severely depressed. Note also that ganglionic blockade has only moderate effect on blood pressure.

Table 1 *Calculated emptying rates of rabbit penis during stimulation with increasing frequencies sympathetic chains and corresponding filling rates during stimulation of the pelvic or hypogastric ne*

	Stimulation frequency Hz						
	0 05	0 1	0 ?	0.5	1	[illegible]	4
A *Average rate to peak of response*							
Symp. chains	0 1?±0 0_	0 17±0 04	0 26±0 07	0.37±0.10	0 53±0.13	0.74±0 07	[illegible]
Pelv nerves	–	–	–	0 15±0.04	0.13±0 03	0 _3±0.05	[illegible]
Hypogastr n	–	–	–	–	–	0 09±0 06	[illegible]
B *Rate during rapid phase of response*							
Symp chains	0 44±0 13	0 95±0._5	0 98±0.19	1 74±0.33	_11±0 08	3 16±0.78	[illegible]
Pelv nerves	–	–	–	0 7±0 04	0.29±0 08	0 5_±0 15	[illegible]
Hypogastr n	–	–	–	–	–	0.16+0 11	[illegible]

Rates in ml/min mean±S E. $n=3$ The calculations are based on the same expts. as Fig. 6 and the left panel of Fig. proper evaluation of the values it should be known that in these expt the volume difference between maximum p due to vasodilation and maximum retraction due to vasoconstriction was 0 35–0 45 ml. Note that at frequencies be the emptying rates were always faster than the filling rates the difference being more pronounced at lower frequ especially during the rapid phase of response. Note also that at frequencie below 8 Hz the filling rates due to stimul the pelvic nerves clearly exceeded those due to stimulation of the hypogastric nerves

penile volume in animals with intact or broken penile vasomotor supply Stimulation (4–3? Hz) of the left splanchnic nerve in animals with intact sympathetic chains produced only faint retractions However if the chains were cut and the penis was protruded clear retractions could be obtained with such stimulations In the most sensitive of the three rabbits in which the right adrenal had been ligated clear retraction was seen with 4 Hz and maximum retraction with 16 Hz (unlimited train stimulation) which corresponded to about 0 ? Hz stimulation of the sympathetic chains Already stimulation with 4 Hz of the splanchnic nerve had marked effects on the blood pressure We conclude that adrenal medullary catecholamine secretion hardly partici pates to any significant degree in normal control of penile volume but could affect it under extreme conditions as e g asphyxia (Sjöstrand & Klinge 1979)

B *Injection of noradrenaline or adrenaline* In rabbits with intact sympathetic chains i a injections of 10–50 nmol/kg of either of these amines had but faint effects on penile volume However if the vasomotor tone had been broken and the penis was protruded prompt retractions were obtained (Fig. 4) I v injections of the above amounts of these amines were almost ineffective In this context it should be mentioned that we never saw any clear protrusion after i.a. injection of adrenaline or nor adrenaline not even after α-adrenoceptor block ade This also holds true for stimulation of the splanchnic nerve or the sympathetic chain. Sin the adrenal of the rabbit contains almost exclu adrenaline (Euler 1956) we conclude that we h not obtained any evidence for a signific physiological role of humoral or innervated β re ceptors in the penile vascular bed although w are present since terbutaline (μmol/kg) produ protrusion in spite of simultaneous fall in bl pressure (cf Klinge & Sjöstrand 1974, 1977a)

Vasodilator innervation s inhibitory nerves of penile smooth muscle (Figs. 6–10 and Table 1)

A *Anatomy and general physiology* Two n sets i e the pelvic and the hypogastric ne were found to convey erectile fibres We found evidence for erectile fibres in the pudic nerve the sympathetic chain at the level of L–S. T concept is based on expts on untreated anima well as on animals treated with guanethidine hydroergotamine phentolamine or phenox zamine and is in disagreement with the re of François Franck (1895) and Bacq (1935) and They found dilator fibres also in these nerves

As expected the erectile responses were de dent on the arterial blood pressure (Fig. 9) also were dependent on the phase of rhythmic lations if such were present i e the standard s lation of ?0 s had much smaller effect if the was spontaneously retracting Severing hypogastric and/or pelvic nerves was without on the resting volume of the penis Thus we

	16	32
	1.25±0.28	0.76±0.15
)	1.15±0.50	1.05±0.58
)	1.05±0.76	0.67±0.41
)	4.06±0.93	2.19±0.73
)	2.67±1.40	2.09±1.21
)	2.72±1.65	1.60±0.80

that there was no significant tonic discharge dilator fibres.

Pelvic pathw 1) *Pharmacology* The dilator nse was blocked by mecamylamine, the doses in general higher than those required to block asomotor tone i.e. 30–60 μmol/kg. This effect best seen n animals in which the sympathetic s had been cut prior to the expt in order to *mize* the decrease of blood pressure (Fig. 7). s our stimulation was preganglionic (cf Lang & Anderson 1895). We did not observe any overt effect of even very large doses of atropine (140 μmol/kg) or scopolamine (250 μmol/kg) in atropine sensitive or semiresistant rabbits on the erectile response to pelvic nerve stimulation (?0 s 0.5–16 Hz). This also applies to propranolol (10 μmol/kg) mepyramine (.5 μmol/kg) cimetidine (40 μmol/kg) and methysergide (4.3 μmol/kg) Further more the dilator response of the selectively per fused penile artery to pelvic nerve stimulation (6–1 Hz, 30 s expts) was not overtly affected even by long time perfusion with .6×10 M scopolamine (Fig. 10c) This concentration of scopolamine blocked the effect of acetylcholine injected into the penile artery (Fig. 10b)

) *Frequency-response relationship* Threshold frequency for a response was 0.1–0.5 Hz. With 0.5 Hz steady and reproducible effects were obtained in most expts Fig. 6 shows a frequency response plot to unlimited stimulation. The expts were per formed on rabbit with intact vasomotor tone Maximum protrusion is obtained within 1– Hz. While it s of interest for the understanding of long lasting erections as may be the case e g. in man the curve gives however but little of the dynamics of erection. The corresponding filling rates are therefore presented in Table 1 in which the calculated a erage as well as maximum rates are given The erectile responses were not distinctly biphasic as were the retractile responses. In general there was a lag phase a short slow phase and a rapid phase which eventually levelled off At increasing fre-

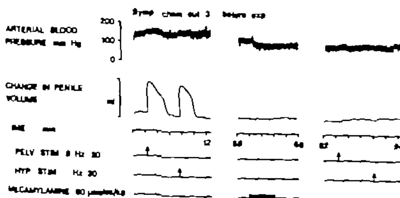


Fig 7 Rabbit, 4.1 kg, urethane, sympathetic chains cut 3 days prior to expt Left panel: Responses to pelvic and hypogastric nerve stimulation. Note size and duration of responses and also rapid phase of retraction Middle panel: Mecamylamine is without significant effect on penile volume (cf Fig 4B) Right panel: After ganglionic blockade the responses to hypogastric and pelvic nerve stimulation are severely depressed. Note also that ganglionic blockade has only moderate effect on blood pressure

Table 1 *Calculated emptying rates of rabbit penis during stimulation with increasing frequency* [illegible] *sympathetic chains and corresponding filling rates during stimulation of the pelvic or hypogastric* [illegible]

	Stimulation frequency Hz						
	0 05	0 1	0.2	0.5	1		4
A *Average rate t peak of response*							
Symp chains	0 1 ±0 0	0 17±0 04	0 .6±0 07	0.37±0 10	0.53±0.13	0.74±0 07	1[illegible]
Pelv nerves	–	–	–	0 15±0.04	0.13±0.03	0 .3±0 05	1[illegible]
Hypogastr n	–	–	–	–	–	0 09±0 06	[illegible]
B *Rate during rapid phase of response*							
Symp chains	0 44±0 13	0 95±0 25	0 98±0.19	1 74±0.33	.11±0.08	3 16±0.7	[illegible]
Pelv nerves	–	–	–	0..7±0 04	0.?9±0.08	0.5.±0 15	1[illegible]
Hypogastr n	–	–	–	–	–	0.16±0 11	[illegible]

Rates in ml/min mean±S E $n=3$ The calculations are based on the same expts. as Fig. 6 and the left panel of Fig [illegible] proper evaluation of the values it should be known that in these expts the volume difference between maximum pr[illegible] due to vasodilation and maximum retraction due to vasoconstriction was 0.35–0 45 ml. Note that at frequencies [illegible] the emptying rates were always faster than the filling rates the difference being more pronounced at lower frequencies especially during the rapid phase of response Note also that at frequencies below 8 Hz the filling rates due to stim[illegible] the pelvic nerves clearly exceeded those due to stimulation of the hypogastric nerves

penile volume in animals with intact or broken penile vasomotor supply Stimulation (4–32 Hz) of the left splanchnic nerve in animals with intact sympathetic chains produced only faint retractions However if the chains were cut and the penis was protruded clear retractions could be obtained with such stimulations In the most sensitive of the three rabbits in which the right adrenal had been ligated clear retraction was seen with 4 Hz and maximum retraction with 16 Hz (unlimited train stimulation) which corresponded to about 0 ? Hz stimulation of the sympathetic chains Already stimulation with 4 Hz of the splanchnic nerve had marked effects on the blood pressure We conclude that adrenal medullary catecholamine secretion hardly participates to any significant degree in normal control of penile volume but could affect it under extreme conditions as e g. asphyxia (Sjöstrand & Klinge 1979)

B *Injection of noradrenaline or adrenaline* In rabbits with intact sympathetic chains i.a injection of 10–50 nmol/kg of either of these amines had but faint effects on penile volume However if the vasomotor tone had been broken and the penis was protruded prompt retractions were obtained (Fig 4) i v injections of the above amounts of these amines were almost ineffective In this context it should be mentioned that we never saw any clear protrusion after i.a. injection of adrenaline or noradrenaline not even after α-adrenoceptor block ade This also holds true for stimulation of the

splanchnic nerve or the sympathetic chains Se[illegible] the adrenal of the rabbit contains almost exclu[illegible] adrenaline (Euler 1956) we conclude that we h[illegible] not obtained any evidence for a signif[illegible] physiological role of humoral or innervated β[illegible] ceptors in the penile vascular bed, although [illegible] are present since terbutaline (? µmol/kg) prod[illegible] protrusion in spite of simultaneous fall in b[illegible] pressure (cf Klinge & Sjöstrand 1974, 1977a)

Vasodilator innervation s inhibitory nerves of penile smooth muscle (Figs 6–10 and Table I[illegible]

A *Anatomy and general physiology* Two [illegible] sets i e the pelvic and the hypogastric n[illegible] were found to convey erectile fibres. We fo[illegible] evidence for erectile fibres in the pudic nerv[illegible] the sympathetic chain at the level of L4–S[illegible] concept i based on expts on untreated anim[illegible] well a on animal treated with guanethidi[illegible] hydroergotamine phentolamine or pheno[illegible] zamine and is in disagreement with the [illegible] of François Franck (1895) and Bacq (1935) o[illegible] They found dilator fibres also in these nerve[illegible]

As expected the erectile responses were [illegible] dent on the arterial blood pressure (Fig. 9[illegible] also were dependent on the phase of rhythm[illegible] lations if such were present i e the standar[illegible] lation of 20 s had much smaller effect if th[illegible] was spontaneously retracting. Severing [illegible] hypogastric and/or pelvic nerves was witho[illegible] on the resting volume of the penis Thu[illegible]

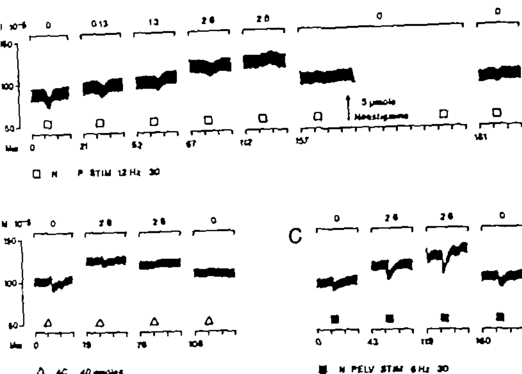


Fig. 10 Effect of scopolamine on dilator responses in the selectively perfused penile artery. *A*. Rabbit, 3.2 kg, urethan, perfusion of right penile artery. The response to hypogastric nerve stimulation is gradually decreased by increasing concentrations of scopolamine but only *after about 30 min perfusion with 2.6 · 10⁻⁸ M* is the response abolished. It was abolished also after switching to scopolamine-free perfusion fluid, but after a large dose of neostigmine injected into the artery the response slowly reappears. Neostigmine by itself produces a transient reduction in resistance. *B*. Rabbit, 2.8 kg, urethan, perfusion of left penile artery. Responses to injected acetylcholine. First panel: Control. Initial slight increase and later decrease in resistance. Second panel: After 15 min of high scopolamine concentration in perfusion fluid a clear response is still left. Third panel: After about 60 min of scopolamine perfusion the response disappeared and remains so after switching to scopolamine-free perfusion fluid (last panel).
C. Rabbit 3.0 kg, *urethan, perfusion of right penile artery*. Decreases in resistance due to pelvic nerve stimulation. First panel: Control. Second panel: After 30 min of scopolamine perfusion the response remains. Third panel: After more than 1.5 h of perfusion with high concentration of scopolamine there is no certain effect on the dilator response; the amplitude is greater, probably merely reflecting the increased vascular tone. Last panel: After switching to ordinary perfusion fluid the response is similar to that in the control record. Note the increase in vascular tone produced by the high concentration of scopolamine seen in all expts.

...educed this response by 50%. The hypogastric ...ble response was not overtly affected by ...anolol (20 µmol/kg), mepyramine (6 µmol/kg), ...idine (4 µmol/kg into the femoral artery) or ...hysergide (4 µmol/kg).

Frequency-response relationship. Threshold ...uency for an erectile response was 0.5–2 Hz. ...ally 2 Hz were needed for steady and reproducible responses. The erectile responses quite ... showed an escape phenomenon, i.e. the penis ...acted in spite of continuous stimulation (2–8 ...). This was rare in the case of pelvic nerve ...ulation. Compared to the pelvicus-induced response the hypogastricus-induced one was more variable and in many expts it was not possible to reach the same level of maximum protrusion as with pelvic nerve stimulation.

In Fig. 6 a frequency-response plot to unlimited train stimulation is presented. The rabbits had intact vasomotor tone. Maximum is obtained within 8–16 Hz. Thus compared to the corresponding curve of pelvic nerve stimulation the hypogastric curve was shifted to the right. The response to hypogastric nerve stimulation was not, however, a right shifted mirror of pelvic nerve stimulation. This can be seen in Table 1 and Fig. 8. The two

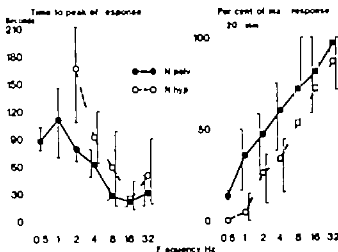


Fig 8 Left panel Frequency-re ponse plots of time in terval between stim. on and peak of erectile response in the same expts as shown in Fig. 6 Each point represents mean and range of 3 expts Note that the curves meet at 16 Hz and that the time interval is about ?0 s at this frequency Right panel Frequency-response relationship of erectile responses to 20 s stimulations of pelvic or hypogastric nerves Each point represents mean and range of 4–9 expts Note that the two curves come closer at 16 Hz. For further explanation of the graphs see text Frequency on logarithmic scale

quencies the response became steeper Thus although maximum protrusion was obtained already with 1–? Hz it took 1–? min to reach it while it was reached within ?0–30 s with 16 Hz In Fig 8 a frequency response plot of estimated interval between start of stimulation and peak response given For comparison a plot of the frequency response relationship of our standard stimulus i e ?0 s is also given When evaluating the curves in Figs 6 and 8 it should be kept in mind that in the rabbit sexual arousal and complete copulation take place within less than ?0 s. This raises the questions in what range do the erectile fibres fire under normal erection and how great are the species differences?

C *Hypogastric pathway* 1) *Pharmacology.* Like the erectile response induced by stimulation of the pelvic nerves also that elicited by stimulation of hypogastric nerves was blocked by mecamylamine (Fig. 7) Consequently also this path has a peripheral ganglionic relay In contrast to the erectile response produced by stimulation of the pelvic nerves that produced by stimulation of the hypogastric nerves showed certain sensitivity to muscarinic blockade i e it could be reduced in atropine sensitive rabbits (Fig. 9) although not abolished Furthermore in one of two expts with selective perfusion of the penile artery the erectile response to hypogastric nerve stimulation (6–1? Hz 30 s) was abolished by scopolamine although long time and high concentrations were needed This was also the case with the effects of acetylcholine injected into the penile artery (Fig. 10b). The hypogastric response reappeared after neostigmine (Fig. 10*a*) In the other exp. scopolamine (2·

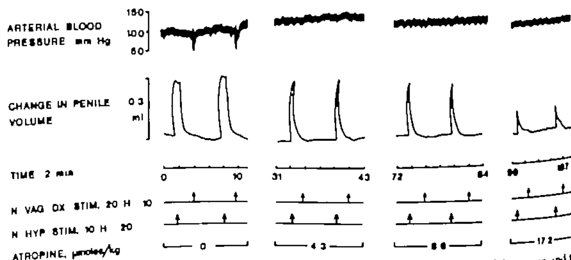


Fig 9 Atropine sensitive rabbit, 4 9 kg, urethan Responses to alternate stimulations of the right vagus and the hypogastric nerves. Suppression of responses by increasing doses of atropine Left panel Control Note the difference of the two responses reflecting the difference in blood pressure Middle-left panel. After a moderate dose almost ... remains of the vagal response The duration of the erectile responses is reduced Note the increase in blood p... Middle-right panel. After doubling the dose of atropine the erectile response are further reduced Right panel fourfold increase in atropine dose there is a severe depression of the erectile response

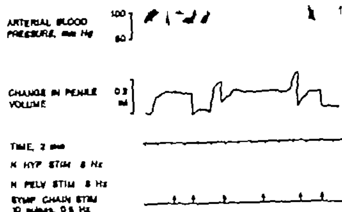


Fig. 12. Rabbit, 4.1 kg, urethan, all nerves to penis cut and placed on electrodes, external iliac vessels tied. The hypogastric nerves are first stimulated until maximum effect is obtained. A short train of pulses at low frequency delivered simultaneously on the sympathetic chains is without effect. After termination of hypogastric stimulation the same short train produces retraction of the penis. Pelvic nerves are then stimulated and penile volume increases again. But this increase is interrupted by retraction when the chains are stimulated. Hypogastric nerves are then stimulated and again simultaneous stimulation of the chains is without effect. Pelvic nerves are then stimulated and the penis reaches greater degree of tumescence than before, yet stimulation of the chains breaks through. Finally after termination of vasodilator stimulation, chain stimulation brings penile olume back to the initial level. Note that pelvic nerv stimulation produces a greater protrusion than hypogastric nerve stimulation. Prior to this tracing the rabbit had been used in the expts. in Fig. 11 B and C.

...lomine had but little effect on blood pres... compared to unsevered controls (Fig. 2) ...e findings were similar in rabbits decentralized ...21 days prior to expts.

...ts of some other biological compound

...ine vasopressin (0.001–0.1 IU/kg) raised the ...l pressure, retracted the protruded penis and ...ressed erectile responses elicited by pelvic or ...gastric nerve stimulation. Similar effects were ...rved with high doses of angiotensin (8–40 .../kg). It is likely that the suppressions of the ...ile responses were due to high resistance in the ...e vascular bed. A very high dose (1 IU/kg) of ...ocin had effects similar to those of vasopressin ...eaker.

...s on the influence of perineal ...ed muscles

...tractions of the perineal muscles encircling the ...a and the bulb of the penis produced distinct ...ts on the recorded penile volume (cf. e.g. ...çois-Franck 1895, Langley & Anderson 1895) ... as clearly seen when the pudic nerve was ...ulated in the absence of decamethonium block ...side. Also reflex contractions sometimes observed during maximum protrusion due to pelvic or hypogastric nerve stimulation increased the recorded penile volume. In one expt the increase was about 50% above maximum protrusion. The volume immediately went back to the preceding level when the muscles relaxed.

DISCUSSION

To the concept that filling of the erectile tissue essentially is due to dilation of it arterial inflow channels which makes the inflow resistance lower than the outflow resistance (Eckhard 1863, Lovén 1866, and later authors as e.g. François-Franck 1895, Henderson & Roepke 1933, Newman et al. 1964, Dorr & Brody 1967) we have nothing to add. We will, however, emphasize the significance of the smooth muscles of the cavernous tissue especially in emptying the penis. This has been somewhat overlooked probably because most studies have been performed on the dog, which has a fairly rigid penis. Several recent studies have illustrated the importance of vasocompression due to contraction of perineal striated muscles in penile erection in many

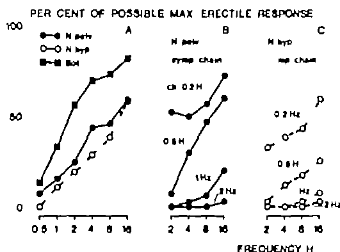


Fig 11 A Synergism between pelvic and hypogastric nerve stimulations. Plots from expts on a 4.3 kg rabbit Frequency-response relation hip to 20 s stimulation Frequency on logarithmic scale Weak dotted line indicates the algebraic sum of pelvic and hypogastric stimulation only Note that the synergism is of type potentiation in the lower frequency range *B* and *C* show plots from expts on a 4 1 kg rabbit where the sympathetic chains were stimulated with indicated frequencies 3 min prior to and during the 20 s stimulation of pelvic and hypogastric nerves respectively Note the gradual suppression of the erectile responses, which are virtually abolished at Hz stimulation of the chain For further explanation see text The erectile responses are calculated in per cent of maximum response possible i e the volume between the actual level of initial retraction and full vasodilator response

response curves converge towards 16 Hz i e the hypogastric curves are steeper suggesting possible differences in neuro-effector mechanisms

Interactions between vasodilator and vasoconstrictor nerves (Figs 11 and 1)

A *Synergism between hypogastric and pelvic nerve stimulations* When both nerve sets were stimulated simultaneously faster and larger responses were obtained than when either set was stimulated alone This was especially clear when standard stimulations were applied In general the synergism was a potentiation in the low frequency range Furthermore subliminal stimulation of the hypogastric nerve could facilitate the response to pelvic nerve stimulation (Fig 11*a*) This figure gives plots from an expt in which the difference between the effects of pelvic and hypogastric nerve stimulation was not very marked

B *Antagonism between vasoconstrictor and vasodilator nerve stimulation* If the sympathetic chains were stimulated simultaneously with the pelvic and/or the hypogastric nerves and at the ... rate the constrictor response strongly dominated More detailed analysis further elucidated this dominance Fig 11*b* and *c* illustrate a representative expt where the sympathetic chain was stimulated 2–3 min prior to and during 20 s stimulation of the pelvic or hypogastric nerves The frequencies applied to the constrictor nerves were one within "normal" penile vasoconstrictor tone i e 0.2 ..., one slightly above it i e 0.5 Hz, and two clearly above it but definitely within what is accepted as physiological range i e 1 and 2 Hz. Already 1 Hz markedly suppresses the erectile responses and at 2 Hz they are essentially eliminated. The strong vaso-constrictor dominance was further substantiated in the reversed type of exp. i e when 20 s stimulations of the chains were applied during continuous stimulation of the pelvic or hypogastric nerve (4 Hz) At 1 Hz or more the constrictor response always broke through and even 0 1 Hz stimulation of the chain could break through 4 Hz stimulation of the dilator nerves A certain difference could in this context be noted between the pelvic and hypogastric nerves Thus in 2 out of 4 expts hypogastric nerve stimulation better counteracted the effect of chain stimulation in the range of ... 0 5 Hz (Fig 12)

Some comments on animals with the sympathetic chains cut prior to expts (Figs 2 and 7)

When the sympathetic chains were cut during operation the penis immediately protruded but after ... h it was retracted again In later expts the penes of these animals were hyperinvoluted as compared with those of unoperated animals These penes were less sensitive to stimulation of the erectile fibres especially to hypogastric nerve stimulation at frequencies up to 8 Hz Small or ... responses or prominent escape phenomena were seen But 8–16 Hz were usually as effective as in controls Since the cutting essentially was preganglionic (see above) and since neither phentolamine (Fig 2) nor mecamylamine (Fig 7) produced protrusion of these penes we conclude that the hyperinvoluted and retracted state was due to increased myogenic tone rather than to increased sensitivity to circulating catecholamines. In this respect the penile vascular bed probably did not diverge from the rest of the vascular beds of the hind-body because on average these rabbits had higher blood pressure ... the others

tanen 1964 Klinge & Penttilä 1969). Also quency-response relationship of the hypogas-nulation and its tendency to escape phenom-nay point to a cholinergic component (cf thoder & Folkow 1953 Folkow Mellander rg 1961 Djojosugito et al. 1968) It may seem dictory to our earlier concept that we here neurogenic muscarinic relaxation of penile k muscle. It is not so however because the rnic relaxation most likely is restricted to the rterial branches and it is known that arterioli laxed by acetylcholine whereas larger vessels ntracted (cf Somlyo & Somlyo 1968, Mellan-Johansson 1968). We have suggested that ergic nerves may contribute to erection by ctional muscarinic inhibition of excitatory ergic neurotransmission (Klinge & Sjöstrand Klinge, Eranko & Sjostrand 1978) The nogram shown in Fig. 1 may support this pt. The strong dominance of the excitatory s points to the value of such a mechanism (see jostrand & Klinge 1979) It further should be oned that a more detailed analysis might have that the difference between the hypogastric he pelvic pathways concerning preganglionic y to different dilator neurons is a quantitative ather than a purely qualitative one Thus it has demonstrated that individual pelvic neurons ive excitatory input from both the hypogastric the pelvic nerve (Crowcroft & Szurszewski). Finally it is also possible that postganglionic nergic hypogastric fibres could via nicotinic ptors stimulate postganglionic non-cholinergic c fibres (cf. Klinge & Sjöstrand 1974 1977*a*)

wish to thank Mrs Annika Rosen and Mr Einan ...ain for excellent assistance in making the figures, Kerstin Westing and Mrs Rosen for excellent and al technical assistance and Mr Jan Svensson for skil-... of the electrodes. The study was supported by from the Magnus Bergvall Memorial Fund and the ish Medical Research Council project number B 76-04733 (to N S) and by scholarship from the Medi-Research Council of the Academy of Finland (to ...)

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mammals (e g Beckett et al 1972 1973) We find it likely that compression of the crura and the bulb and a thereby produced distal displacement of blood which enlarges straightens and stiffens the penis could be of great importance in the brief copulation of the rabbit Paralysis of striated muscles is possibly one explanation for the im paired erection observed in the rat after section of the pudic nerve (Lodder & Zeilmaker 1976)

Obviously penile tumescence as well as de tumescence can be elicited from various levels of the CNS including the spinal cord alone (e g. Kuhn 1950 Thielen et al 1969) but we will confine this discussion to the principal mechanisms which in the periphery regulate the penile volume Two main mechanisms can keep the penis in its usual pro- tected flaccid and retracted state i e the vaso- motor tone and the automaticity of penile smooth muscle The exceptionally low adrenergic tone needed to keep the penis withdrawn is in good agreement with our previous findings on isolated smooth muscle effectors of penile erection concern ing their sensitivity to excitatory nervous stimuli and noradrenaline (Klinge & Sjöstrand 1974 1977*a*) In case of regulation of penile volume also the functional geometry of the muscles deserves attention The deep penile artery has ridge-like thickenings formed by longitudinal smooth muscles projecting into the lumen the helicine branches have thick muscular walls and in the final branches there are myoepitheloid like elements (vide Fuji moto & Takeshige 1975) Hence the wall/inner radi- us ratio (cf Folkow & Neil 1971) will be very high and even slight contraction of the smooth muscles will produce very great resistance to inflow Con cerning the cavernous spaces the capacitance sec tion the stored volume of blood will depend roughly on the second or the third power of the inner circumference of the wall and the mass of the compressed trabecular tissue Thus also in this compartment there will be a considerable geometric reinforcement of the effects of variations in smooth muscle length Furthermore the production of vasodilator metabolites may be very low in an organ composed of smooth muscle and connective tissue

Our results indicate that quite rapidly after re moval of the vasoconstrictor tone autoregulation takes over and the final outcome seems to be hyperinvoluted penis rather than a tumescent one This may be the natural consequence of replace ment of the superior control exerted by vasomotor tone by local control due to pacemaker cell a[illegible] opposed by just a minute metabolic [illegible] feedback

Three autonomic mechanisms which eithe[r] or in combination could produce penile tume[scence] were found i e 1) interruption of the alre[ady low] vasomotor tone 2) discharges in the [pelvic] parasympathetic nerves and 3) discharges [in the] sympathetic hypogastric nerves The fir[st has] already been touched Alone it seems to [be a] somewhat variable unsafe and insuffi[cient] mechanism The second mechanism is evident[ly the] most efficient one since the pelvic nerves ca[n oper]ate at very low frequencies In this respect [it re]sembles the non-cholinergic inhibitory fibres [which] we have studied in isolated smooth muscle effe[ctors] of penile erection (Klinge & Sjöstrand 1974 19[77]) This similarity along with the resistance [to] atropine and scopolamine prompts us to su[ggest] that the pelvic nerves mainly convey pregan[glionic] fibres to non-cholinergic inhibitory neuro[ns in]nervating penile smooth muscles

Concerning the third mechanism we feel now more than a hundred years after their o[riginal] description (Eckhard 1876) it is time to [generally] accept the existence of the sympathetic hypog[astric] erectile fibres The reason is not only the [direct] demonstration of their existence but also the [work] of Müller (1906) and Root & Bard (1947) on [dogs] and cats respectively which clearly show [that] animals with the sacral cord removed still can [have] erections and copulate These erections [are] abolished if the chordotomy is extended to the [low]er thoracic region or if the lumbar sympat[hetic] chains are removed Furthermore there are se[veral] clinical reports showing that men with seve[re le]sions in the lower lumbar or the sacral cord [or] cauda equina can have psychogenic erect[ions] although they may have full paralysis of the [bladder] and the perineal striated muscles and no re[flex]ogenic i e local erections (e g Bernhardt [1888] Thorburn 1888 Kuhn 1950 Bors & Comarr [1960] Tsuji et al 1961 Comarr 1970 Gunterberg & [Peter]sén 1976) The interpretations of the mecha[nisms] of these erections become very strange if only [the] parasympathetic pathway for erection is accep[ted]

Our pharmacological results suggest that a[lso] in some rabbits the hypogastric nerves may [also] carry preganglionic fibres to cholinergic vasodi[lator] neurons Acetylcholine is present in the rabbit p[enis] which has cholinesterase positive nerves (Per

renergic and novel non-adrenergic neuronal chanisms in the control of smooth muscle ivity in the human oviduct

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The isthmic portion of human Fallopian tubes was excised during surgery and the ampullary-isthmic junction was identified The external, longitudinal and the inner circular muscle layers of this structure were then mechanically separated. The contractile activity of thin strips from these layers was studied isometrically in organ bath experiments. Administration of various adrenoceptor agonists and antagonists revealed that the responses of the longitudinal smooth muscle are primarily mediated by α-adrenoceptors while there is marked dominance of β-adrenoceptor-mediated effects in the circular layer Transmural nerve stimulation caused neurogenic responses similar to those caused by exogenous noradrenaline although nerve-mediated inhibition of circular muscle activity was not abolished entirely by propranolol, atropine or guanethidine, but by tetrodotoxine indicating the existence of a hitherto unknown a non-adrenergic, non-cholinergic, inhibitory neurogenic mechanism. The results support the concept of specific sphincter like function controlling ovum transport at the human oviductal isthmus, and that this sphincter may be controlled by both adrenergic nerves and nerves liberating an unidentified transmitter substance

Key ords Oviduct, human, smooth muscle, nerves, adrenergic

ent studies of the normal time course of gamete sport through the human oviduct have demonstrated that the ovum reaches the proximal part the ampulla within a few hours after ovulation, remains there for 2 to 3 days before its rapid nsit through the isthmus (Chevrakoff et al. 1976). sphincter-like function may therefore be attributed to the ampullary-isthmic junction (AIJ) a structure which is abundantly innervated by adrenergic fibres (Brundin & Wirsén 1964 Owman et 1967).

The contractile responses of the human oviduct catecholamines have been investigated previously by Sandberg et al. (1960) who used whole segments of the tube to record motility along its long and found that both noradrenaline (NA) and renaline (A) elicited stimulatory effects. Rosenbaum et al (1966) recorded resistance to intraluminal perfusion and were the first to clearly demonstrate the existence of both α- and β-adrenoceptor-mediated responses in the human oviduct. The AIJ was identified by Seitchik et al (1968) who used a hydrostatic model with estimation of opening pressure but the effects of NA on this system were inconsistent. At the human AIJ there are two prominent muscle layers an external layer rich in collagen and blood vessels with predominantly longitudinally orientated muscle fibres, and an inner circular or spiral-shaped layer (David & Czernobilsky 1968 Pauerstein et al 1968) The aim of the present study was to elucidate the adrenergic responses of oviductal smooth muscle by use of an in vitro method allowing selective recordings of circular and longitudinal muscle activity

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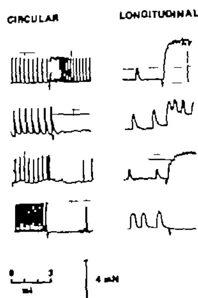
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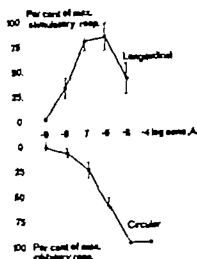


Fig. 4. Cumulati e concentration–effect curves for adrenaline. The maximal stimulatory (longitudinal strips) and inhibitory (circular strips) responses occur at different concentrations. Mean ±S E.

… Typical tracings showing the effects of various …noceptor agonists. *PE*, phenylephrine 10 μm; *NA*, …drenaline 3.0 μM, *A*, adrenaline 3.0 μM, *IP* iso…aline 0.03 μM. Note the differentiated responses of …wo muscle layers to noradrenaline and adrenaline i.e. …ibition of circular preparations and stimulation of …tudinal preparations.

…nt' *t*-test was applied for the comparison of groups. …value of less than 0.05 was considered significant.

…ULTS

…ntaneous contractile acti ity. After the tissues …been mounted in the organ baths both circular …longitudinal preparations developed spontane… …phasic activity within 20 min (Fig. 1). The …raction frequency was consistently higher in …ular strips whereas the duration of each con…tion was greater in longitudinal specimens. The …traction amplitude did not differ significantly in …two types of preparations. Fig. 2 illustrates the …ntitative differences in frequency duration and …plitude between the two types of specimens. In …absence of drug administration, the spontane… activity was most often found to continue for …h. However experiments involving drug ex…sure or nerve stimulation were limited to the first …h.

…ffects of adrenoceptor agonists. The adminis…tion of the α-adrenoceptor agonist phenylephrine in concentrations ranging between 0.03–100 μM caused a dose-dependent stimulation of longitudinal muscle activity while there was a very weak and temporary increase in contraction frequency of circular specimens, appearing solely at the highest concentrations (10–100 μM) applied (Fig. 3). Noradrenaline (NA) and adrenaline (A) resulted in concentration-dependent inhibition of circular strips and in clear-cut stimulation of longitudinal preparations. The non-selective β-adrenoceptor agonist isoprenaline (0.01–0.3 μM) caused reversible inhibition in both circular and longitudinal specimens.

The concentration–effect curve for A (Fig. 4) illustrates that the stimulatory response in longitudinal specimens is elicited at lower concentrations than the inhibitory response in circular preparations.

H 133/22, a substance which has a high specificity for β_1-adrenoceptors (Carlsson et al. 1977) inhibited contractility in longitudinal specimens at 1.0 μM and in circular preparations at concentrations of 3–10 μM. On the other hand, the β_2-selective agonist terbutaline inhibited contractile activity in circular strips at lower concentrations (0.3–1 μM) than in longitudinal preparations (3–10 μM) (Fig. 5).

Effects of nerve stimulation. Transmural field stimulation (TNS) (4–6 Hz, ~4 ms supramaximal voltage) resulted in an inhibition of the circular muscle activity and stimulation of longitudinal

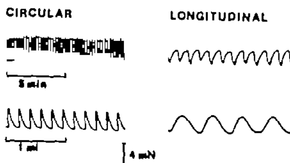


Fig 1 Spontaneous rhythmic activity of strips isolated from the circular and longitudinal muscle layers at the ampullary-isthmic junction The lower tracings show contractions recorded at higher paper speed Note the difference in contraction frequency and duration between circular and longitudinal specimens and the regularity of contractions

Some of the present results have earlier been presented in preliminary form (Lindblom et al 1978*a* 1979)

MATERIAL AND METHODS

Subjects Tissues from 34 women 27–41 years of age were included All patients had regular menstrual cycles and hormonal contraception had been withdrawn for at least months prior to bilateral tubal resection Routine premedication (mepiridine hydrochloride 50 mg, prometazine dichloride 25 mg, atropine sulphate 0.5 mg) and general anesthesia were given to all patients Following opening of the abdomen, 4–5 cm of the medial portions of the tubes were rapidly excised without clamping in order to avoid warm ischemia and immediately placed in chilled oxygenated buffer (composition see below) The specific phase of the menstrual cycle was estimated by histological examination of an endometrial biopsy and by the serum levels of LH progesterone and estradiol 17β just prior to surgery All hormone analyses were performed by radioimmunoassays

Preparation of strip The AIJ was identified by introducing a 1 5 mm wide probe into the distal segment of the tube until a resistance was felt (for further details see Lindblom et al 1978*b*) The peritoneum was carefully removed by use of micro-scissors, exposing smooth muscle fibres with predominantly longitudinal orientation Longitudinal muscle strips were dissected and the remaining circular muscle was incised along its long axis and transversely into strips. Both circular and longitudinal strips were approximately 4 mm long with cross sectional areas of approximately 1 mm^2

Recording of contractile activity One strip of longitudinal and one of circular smooth muscle were mounted in the same organ chamber between separate hooks and force transducers (Grass model FT 03) [illegible] The bath contained 50 ml of buffer solution [illegible] 37°C and the solution was continuously aerated with a mixture of 96% O_2–4% CO_2, at a constant pH [illegible] 7.34±0.04 Contractile activity was recorded [illegible] at a passive force of 4 mN on a Grass (model 7) polygraph The preparations were allowed to accommodate [illegible] applied passive tension for 45–60 min before exposure to drugs or transmural field stimulation (TNS) The mechanical response to a certain stimulus was [illegible] tated by use of electronic integration or [illegible] mean force during influence of the stimulus after [illegible] tion of the mean force of the spontaneous activity [illegible] mediately prior to the application of the stimulus

Buffers and chemical Oxygenated Krebs [illegible] bicarbonate buffer was used for the dissection and [illegible] tified with 10 0 mM *d*-glucose for the contractility [illegible] The composition of the buffer was (mM): NaCl [illegible] 4 7 $CaCl_2$ 2.5 $MgCl_2$ 1 19 $NaHCO_3$ 15.5, KH_2PO_4 1 (*d* glucose 10 0) In expts including drug exposure [illegible] nerve stimulation EDTA was added to the buffer [illegible] to give a final concentration of 0.026 mM The following drugs were used phenylephrine (Sigma Chemical Company Mo USA) noradrenaline bitartrate (Noradrenalin [illegible] Apotekbolaget Ltd Sweden) adrenaline bitartrate (Adrenalin ACO Ltd. Sweden) isoprenaline chloride (Sigma Chemicals) terbutaline sulphate (Bricanyl® Draco [illegible] Sweden) phenoxybenzamine hydrochloride (Dibenzyline SK & F Laboratories Ltd U K) propranolol chloride (Inderal® ICI Ltd U K) atropine sulphate (Atropin ACO Ltd Sweden) tetrodotoxine (Sigma Chemicals) 133/22 (prenalterol) (Hassle Ltd Sweden), EDTA sodium calcium salt (Sigma Chemicals) guanethidine sulphate (Ciba-Geigy AG Switzerland)

Statistical procedures Mean values ± S.E. are pr[illegible]

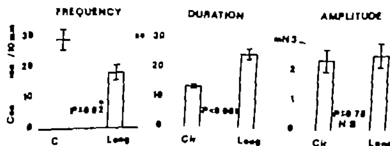


Fig 2 Diagrammatic illustration of frequency duration and amplitude of contractions in circular and longitudinal preparations Mean ± S E n=34

CUSSION

...se of small muscle specimens carefully dis...ed from the two perpendicular muscle layers of ...oviductal isthmus ascertains experimental ad...ages compared to previous studies on this or... here comparatively large sections have been ...in vitro (e.g. Moawad et al. 1976). Thus, the ...rent properties of the spontaneous, myogenic ...vity of the two muscle layers could be compared ... an interesting qualitative differentiation of ...noceptor-mediated responses between the ...hanically separated outer longitudinal and the ...r circular muscle could be identified. Fur...more the relatively small size of the prepara...s used in the present study reduced the risk of ...cting inadequate responsiveness of the tissue ... to improper oxygenation, as may be the case in ...e isolated specimens (cf. Hellstrand 1978).

...me interesting finding was the consistently low...ude and greater duration of the phasic contrac...s of the longitudinal muscle compared t... the ...ular one in spite of comparable degrees of ...tch, cross sectional area, passive force and ...gth of preparations. This strongly indicates dif...entiated smooth muscle membrane properties of ... two layers of effector tissue in the human Fallo...n tube.

...n the longitudinal smooth muscle sympathetic ...ve stimulation or exogenous administration of ... or A invariably caused an α-adrenoceptor ...diated excitatory response whereas β-adreno...tor mediated inhibitory responses were obtained ... the circular muscle layer. Apparently this dif...entiation of responses reflects a variable domi...nce of α- and β-adrenoceptors in the two tubal ...ers.

...Although the present results may not be sufficient ... a detailed sub-classification of the β-adrenocep...s into the β_1- and β_2-types according to Lands ...ands et al. 1967) it is of interest that terbutaline ...ting mainly on β_2-receptors, caused inhibitory re...onses in circular specimens at lower concentra...ons than did H 133/22, which has a higher affinity ...r β_1-adrenoceptors (Carlsson et al. 1977).

As to the physiological significance of the func...onal organization of the isthmic portion of the ...man oviduct it is reasonable to consider that this ...tory segment may indeed exert a sphincter like ...nction. The morphological distribution of sym...pathetic fibres within the isthmus (Owman et al. 1967) forms the structural basis for an adrenergic control mechanism. Furthermore, the functional properties including the varying adrenoceptor sensitivities of the two muscle layers found in the present experiments suggest that such a sphincter could be comprised of two functionally different mechanisms corresponding to the circular and longitudinal smooth muscle layers, respectively. Accordingly increased adrenergic nerve activity would lead to a relaxation of the β-adrenoceptor dominated circular layer concomitant with a stimulation of the α-dominated longitudinal muscle. In consequence the resultant simultaneous widening of the lumen and shortening of the tube would facilitate the passage of the ovum. The sensitivity of the α- and β-adrenoceptors in this region has been claimed to be influenced by both estrogens and progesterone (Moawad et al. 1976, Owman et al. 1976). However the present study did not reveal any qualitative differences in adrenoceptor responses during the menstrual cycle although minor quantitative differences cannot be excluded to date.

The fact that nerve-mediated inhibition could not be blocked by propranolol, atropine or guanethidine but was abolished by TTX provides strong evidence for the existence of a previously unknown neurotransmitter in human oviductal smooth muscle. A non-adrenergic inhibitory transmitter has also been suggested to operate in the rat oviduct (Murcott & Carpenter 1977) indicating that a novel non-adrenergic, inhibitory neurogenic mechanism may have general applicability.

In a brief report Sjöberg et al. (1978) demonstrated by use of immunofluorescence techniques, that both substance P and vasoactive intestinal peptide (VIP) occur in nerves of the female urogenital tract. In view of these findings it is tempting to suggest that some peptide indeed acts as an inhibitory transmitter in the human Fallopian tube.

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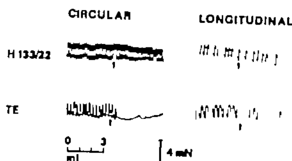


Fig 5 The influence of H 133/22 a "β_1 selective agonist and terbutaline a "β_2-selective agonist on the contractile activity of circular and longitudinal muscle specimens. The concentration of both agonists is 1 0 μM Note the weak inhibitory response of H 133/22 in longitudinal strips and the marked inhibition caused by terbutaline in circular preparations

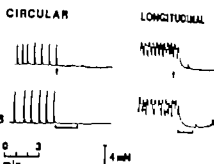


Fig 7 The effect of treatment with the ... phenoxybenzamine (0 1 μM) on responses to NA and TNS (compare Figs. 3 and 6) The stimulatory effects in longitudinal strips are changed into inhibition and the inhibitory responses of circular strips are further pronounced by phenoxybenzamine.

specimens (Fig. 6) Addition of guanethidine (5 μM) to the medium 15 min prior to TNS abolished the stimulatory response of longitudinal muscle strips and frequently an inhibitory response occurred However guanethidine only marginally reduced the inhibitory response of circular strips to TNS Pretreatment with the nerve blocking agent TTX (0.5 μM) abolished both the inhibitory response of circular strips and the stimulatory response of longitudinal specimens but did not influence the effects of NA

Influence of adrenoceptor antagonists Pretreatment with the α adrenoceptor blocking agent phenoxybenzamine (0 1 μM) reversed the stimulatory response of longitudinal preparations to NA and TNS into an inhibition and augmented the inhibitory effects of these stimuli on circular strips (Fig. 7) Furthermore phenoxybenzamine blocked the stimulatory effects of phenylephrine

The inhibitory response of circular strips to NA was abolished by pretreatment with the β-blocking agent propranolol (3–12 μM) for at least 30 min and occasionally a stimulatory response was observed (Fig 8) Propranolol also completely abolished the inhibitory effects of isoprenaline On the other hand the inhibitory response of circular strips to TNS could not be completely blocked by propranolol The stimulatory responses of longitudinal specimens to NA and TNS were further augmented by propranolol

There was no qualitative difference in response to agonists or TNS in specimens obtained at different phases of the menstrual cycle

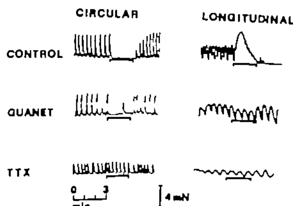


Fig 6 Effects of TNS (4 Hz 2 ms 18 V—) on circular and longitudinal specimens (upper tracings) After treatment with guanethidine (5 μM) the stimulatory response of longitudinal strips is abolished (middle right) In circular strips the inhibitory response to TNS is only marginally reduced by guanethidine (middle left) In presence of TTX (0.5 μM) TNS is uneffective in both circular and longitudinal preparations (lower tracings)

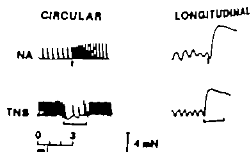


Fig 8 After β-blockade with propranolol (12 μM) the inhibitory response of circular strips to NA is shifted to stimulation and the stimulatory effects of NA and TNS on longitudinal preparations are increased However the inhibitory response of circular strips to TNS is not abolished by propranolol

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alysis of the length response to a force step smooth muscle from rabbit urinary bladder

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Responses to isotonic quick release of AC stimulated smooth muscle strips from rabbit urinary bladder were analysed. Releases were performed at the peak of contraction and at preset tension level in the contraction and relaxation phase. In other expts. responses at 37°C and 27°C were compared. The length response alway consisted of 3 parts. (1) elastic recoil, (2) rapid length change (isotonic transient), (3) steady length change Qualitatively phases (1)–(3) could be distinguished also in responses to isotonic quick stretch. The immediate elastic recoils, phase (1), were described by exponential stress-strain relations. Stiffness was found to be somewhat lower during relaxation than during contraction. N effect of temperature on the elastic recoil was seen. The initial elocity in phase (2) was 2 3 times greater than the velocity 100 ms after release By means of computer analysis of the length records during phases (2) and (3) two decaying exponential processes with widely different time constants could be separated. The time constant of the faster process as of the order of 15–30 ms at 37°C. It decreased with increasing force steps and with increasing temperature. The amount of shortening associated with this proces was correlated with the size of the force step, reaching maximum of about 1.2% of the muscle length. The shortening velocities in phase (3) measured 100 ms after release were described by Hill equation. V_{max} in the rising part and at the peak of contraction were 0.7 and 0.6 L/ respectively at 37°C. Lower values were found during relaxation and at 27°C. We suggest that part of the elastic recoil in phase (1) occurs in structures associated with the individual cross-bridges, that phase (2) is dominated by change in the distribution of conformations of bridges in the attached position and that the shortening rate in phase (3) is determined by the entire cycle of events during turnover of bridges after the muscle has adapted to the new load. Observations on the force response to length steps and on shifts from isometric to afterloaded isotonic contraction and vice ersa are consistent ith this interpretation.

Key ords. Smooth muscle mechanics, elastic recoil, velocity transient, effect of temperature force–velocity relation, cross-bridge theory of contraction

ogress in the understanding of smooth muscle chanics has been relatively slow due to the comx properties of the tissue However with increasing knowledge of the general characteristics of mmalian smooth muscles it became possible to dy their mechanical properties under controlled perimental conditions so that reproducible and liable measurements could be made (for ref see & Murphy 1976, 1979 Johansson 1975) The re onses were analysed in terms of the muscle model f A. V Hill (1938).

In the meantime, observations have been made in eletal muscle which cannot be accounted for by e classical model of series elastic and contractile elements Particularly the analysis of responses to step changes n length or force studied at high time resolution have prompted the development of new concepts (e g. Civan & Podolsky 1966, Huxley & Simmons 1971 Julian Sollins & Sollins 1972, White & Thorson 1973). Therefore, it has become of obvious interest to improve the time esolution of recordings also from smooth muscle to see whether mechanical events in conflict with the classical muscle model would then be revealed in this tissue as well. Research in this direction has been initiated in a few laboratories working with different types of smooth muscle (Johansson, Hellstrand & U ellus 1978, Meiss 1978, Mulvany 1978 Peterson 1978) In

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was elicited at different stages of the isometric contraction. After the accommodation period the stimulus parameters were adjusted so that the preparation gave reproducible isometric contractions of maximal amplitude. Contractions were elicited at intervals of 2–4 min in different expts. A series of isotonic releases to different loads < isometric force were then performed in random order. Releases at the peak of the isometric response were triggered from the timer unit. In the rising and falling phase of the contraction releases were triggered from the force signal when it reached a preset force level on its ascent or descent, respectively. A force baseline was recorded on each frame. The isometric force preceding the load step and the force level to which the muscle was released were measured on magnified projection of the film as a check on the size of the force step calculated in the computer analysis of the digital records (see below).

A second series of expts. was performed to study the effects of temperature. After the accommodation period, perfusion of the bath was switched to 27°C solution. Stimulation of the muscle was started and adjusted to give reproducible contractions of maximal amplitude. A series of isotonic releases from the peak of the isometric response to different loads <P were performed in random order followed in some of the expts. by a series of isotonic stretches to gradually increasing loads >P. The responses were recorded as described above. When enough data had been collected at 27°C perfusion was changed to 37°C solution. Stimulus parameters were adjusted to give reproducible isometric contractions of about the same amplitude as at 27°C. In most cases this adjustment implied an increase in AC frequency. Timer delay was reduced so as to trigger retraction of the lever clamps at the peaks of the faster responses at 37°C. A new series of releases and stretches was run using as nearly as possible the same loads as at the low temperature.

At the end of each expt. the length of the preparation in the clamped position was measured by means of a dissecting microscope with an ocular scale. The muscle was then removed, cut away from the aluminium attachments, blotted and weighed on a Cahn electrobalance. The cross-sectional area was calculated from length and weight assuming a muscle density of 1.05 mg/mm³.

Analysis of data

The starting point for this investigation was the observation that the shortening velocity in smooth muscle was not constant after the initial elastic recoil following a step decrease in load ("quick release", cf. Fig. 2). Instead, the shortening response started at a high velocity, slowed down appreciably during the first 100 ms or so and proceeded with a more gradual decline of velocity thereafter (Johansson, Hellstrand & Uvelius 1978).

In the present paper we have sought a method to study the velocity transient from a single shortening record. If the steady shortening, after decay of the transient, had occurred at a constant velocity this task would be easy. However, in view of the fact that our releases were performed on the rising part of the length-tension curve in order to avoid the high ... at longer ... one can expect, a priori, that shortening velocity will decline during the course of shortening. The following considerations explain the point. Assume that P is linearly related to length (L) over the length interval considered (this was found experimentally to be a very reasonable approximation) and, further that the level of activation is constant over the time interval. Then

$$P_i(L)=P^0+c(L-L^0),\tag{1}$$

where P^0 is isometric tension at the reference length L^0 and c is a constant ($c>0$). The isotonic shortening velocity of the fully adapted contractile element when load is reduced to $P<P^0$ is given by Hill's (1938) equation:

$$\frac{dL}{dt}=V=b(P^0-P)/(P+a).\tag{2}$$

It was found by Uvelius (1977) that, for declining P^0 in rabbit bladder as muscle length was reduced, eq. (2) applies with constant values of a and b. We may therefore expect (2) to be valid at all the different lengths reached by the muscle in the course of shortening. Substitution of $P_i(L)$ from (1) gives

$$\frac{dL}{dt}+\frac{b\times c}{P}L=b\,\frac{L-P_0\ P}{P+a}$$

With the initial condition $L=L^0$ at $t=0$ the solution is

$$L(t)=L^0-\frac{P^0-P}{c}\left[1-\exp\left(-\frac{b\times c}{P+a}t\right)\right].\tag{3}$$

According to (3) we expect the steady shortening response to represent an exponential decay with lower amplitude and rate constant the higher the afterload P. Since also the early transient component of shortening appeared to be exponential in shape (cf. Fig. 4 of Johansson et al. 1978) we considered it reasonable to treat the total shortening response, excluding the series elastic (SE) recoil, as a sum of two decaying exponentials.

$$L(t)=L^0+A_1[1-\exp(-t/\tau_1)]+A_2[1-\exp(-t/\tau_2)]\tag{4}$$

(for shortening A_1, $A_2<0$).

The curve-fitting was carried out on an Alpha-LSI minicomputer equipped with a Tektronix 4010 graphic terminal and 4662 digital plotter. The length and tension records were fed from the tape recorder into an analog-digital converter. Data were sampled at a frequency of 1 kHz. The size of the force step was calculated from the tension data. It would seem desirable to extend the curve fitting of the length records over a long time interval, particularly for recordings at low temperature. Analyses of some of the temperature experiments over both 250 and 500 ms gave very similar results. The final computation in the temperature experiments was based on 90 points, representing the first 500 ms after release. The first 50 points (0–100 ms) were formed as the means of 2 consecutive sample values. The final 40 points (100–500 ms) were formed as means of 10 consecutive sample values. In the experiments comparing the rising and the falling phases of

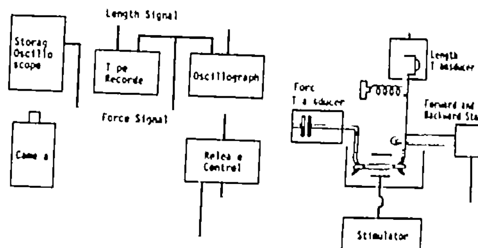


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The present study is concerned with quantitative aspects of this transient in urinary bladder smooth muscle The influence of the size of the load step of variations in temperature and of the time for the release within the contraction–relaxation cycle has been examined

METHODS

1 *Preparation and apparatus*

Subserosal trabeculae of longitudinal smooth muscle from urinary bladder of stunned rabbits were dissected as described by Uvelius (1976) The tissue was trimmed to an appropriate width and a strip of aluminium foil was wrapped around each end of the 3–7 mm long preparation This was then mounted horizontally in a 2 ml organ bath between the arm of a capacitance force transducer and an isotonic lever both of which had conic tips fitting firmly into holes in the pieces of aluminium foil (Fig 1) Tris-buffered Krebs solution was continuously perfused through the bath at a rate of 1 2 ml/min The muscle was stretched to a low preload (<0.3 mN) and allowed to accommodate at 37°C for about an hour

The Krebs solution had the following composition in mM NaCl 120 KCl 6.0 $MgCl_2$ 1.2 CaCl 2.5 glucose 11.5 and tris-(hydroxymethyl) aminomethane (Trizma Base Sigma Chemical Co) 23 It was titrated with HCl to a pH of 7 4 at 37°C or at 27°C when solution of this temperature was to be used The solutions were bubbled with O_2

The capacitance force transducer had a compliance of 0.2 µm/mN a natural frequency of 620 Hz and a sensitivity of 0.1 V/mN The equivalent mass of the isotonic lever at the point of muscle attachment was 8 mg and the sensitivity of the photoelectric transducer recording lever movement was 10 V/mm change of muscle length The compliance of the lever in the clamped position was [illegible] µm/mN

No damping system for the lever other than the bath fluid was provided The load on the lever could be varied from 0 to 30 mN Its position could be clamped by a forward and a backward stop (Fig. 1) which were adjusted by micrometers and could be retracted individually or together by electromagnetic relays The movement of the clamps could be set to give free isotonic releases or controlled length steps For further details concerning the mechanical apparatus see Sjölin Hellstrand & Clemens (1978) Two platinum electrodes in the bath were used to activate the muscle by AC field stimulation (cf Pedersen Laven & Ehl 1975) The specially built stimulator delivered AC stimuli with frequency 25–100 Hz amplitude –4 V and duration 2 5 A timer unit permitted automatic stimulation of the muscle at regular intervals

The force and length signals were displayed on a storage oscilloscope (Tektronix 5103N) and recording was done on film (Grass kymograph camera) magnetic tape (Racal Thermionic Store 4 Instrument tape recorder) and paper (Devices oscillograph MX4) (Fig 1) The start of the AC stimulation triggered a slow sweep of the force record on the oscilloscope allowing the full time course of the contractile response to be observed Release of the isotonic lever triggered a fast sweep of the force and length signals on the oscilloscope

2 *Experimental protocols*

One series of experiments carried out at 37°C was designed to examine the response of the muscle to quick

sponses elicited at different stages of the isometric contraction. After the accommodation period, the stimulus parameters ere adjusted so that the preparation ga reproducible isometric contractions of maximal amplitude. Contractions are elicited at intervals of 2–4 min in the different expts. *A series of isotonic releases to differ* nt loads < isometric force ere then performed in random order. *Releases at the peak of the isometric re*sponse were triggered from the timer unit. In the rising and falling phase of the contraction, releases were triggered from the force signal when it reached preset tension level on its ascent or descent, respectively. A zero force baseline as recorded on each frame. The isometric force preceding the load step and the force level to which the muscle was released ere measured on magnified projection of the film as a check on the size of the load step calculated in the computer analysis of the load records (see below).

A second series of expts. was performed to study the effects of temperature. After the accommodation period, perfusion of the bath was s itched to 27°C solution. Stimulation of the muscle was started and adjusted to give reproducible contractions of maximal amplitude. A series of isotonic releases from the peak of the isometric response to different loads <P were performed in random order followed in some of the expts. by a series of isotonic stretches to gradually increasing loads >P_o. The responses were recorded as described above. When enough data had been collected at 27°C, perfusion was changed to 37°C solution. Stimulus parameters were adjusted to give reproducible isometric contractions of about the same amplitude as at 27°C. In most cases this adjustment implied an increase in AC frequency. Timer delay was reduced so as to trigger retraction of the lever clamps at the peaks of the faster responses at 37°C. A new series of releases and stretches was run using as nearly as possible the same loads as at the low temperature.

At the end of each expt. the length of the preparation in clamped position was measured by means of dissecting microscope with an ocular scale. The muscle was then removed, cut away from the aluminium attachments, blotted and weighed on a Cahn electrobalance. The cross-sectional area was calculated from length and weight assuming muscle density of 1.05 mg/mm³

Analysis of data

The starting point for this investigation was the observation that the shortening velocity in smooth muscle was not constant after the initial elastic recoil following step decrease in load ("quick release" cf. Fig. 2). Instead the shortening response started at high velocity slowed down appreciably during the first 100 ms or so, and proceeded with a more gradual decline of velocity thereafter (Johansson, Hellstrand & Uvelius 1978).

In the present paper we have sought method to identify the velocity transient from a single shortening record. If the steady shortening, after decay of the transient, had occurred at constant velocity this task would have been easy. However in view of the fact that our experiments are performed on the rising part of the length-tension curve in order to void the high lengths one can expect, priori, that shortening velocity will decline during the course of shortening. The following considerations explain the point. Assume that P is linearly related to length (L) over the length interval considered (this was found xperimentally to be very reasonable approximation) and, further that the level of activation is constant over the time interval. Then,

$$P_0(L)\quad P' \quad c(L-L') \tag{1}$$

where P' is isometric tension at the reference length L and is a constant (>0). The isotonic shortening velocity of the fully adapted contractile element when load is reduced to $P<P$ is given by Hill' (1938) equation:

$$\frac{dL}{dt}\quad v = b(P \quad P)/(P+a). \tag{2}$$

It was found by Uvelius (1977) that, for declining P in rabbit bladder as muscle length was reduced, eq. (2) applies with constant values of a and b. We may therefore expect ( ) to be valid at all the different lengths reached by the muscle in the course of shortening. Substitution of $P_0(L)$ from (1) gives

$$\frac{dL}{dt}\quad \frac{b}{P}\quad L = b\quad \frac{L\ -P'_0\ \ P}{P}$$

With the initial condition $L=L$ at $t=0$ the solution is,

$$L(t)\quad L\quad \frac{P'-P}{}\left[1\quad \exp\left(\frac{b}{P}\ c\ t\right)\right] \tag{3}$$

According to (3) we expect the steady shortening response to represent an exponential decay with lower amplitude and rate constant the higher the afterload P. Since also the early transient component of shortening appeared to be exponential in shape (cf. Fig. 4 of Johansson et al. 1978) we considered *it reasonable to treat the total shortening* response, excluding the series elastic (SE) recoil, as a sum of two decaying xponentials.

$$L(t)\quad L\quad A_1[1\quad \exp(-t/\tau_1)]\quad A_2[1\quad \exp(-t/\tau_2)] \tag{4}$$

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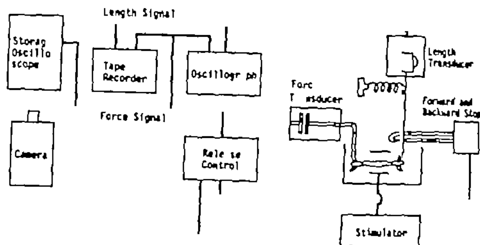


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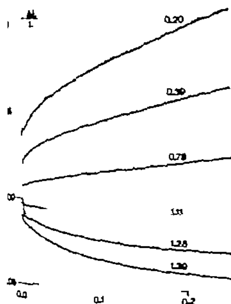
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[illegible] 1. Mechanical parameters at peak and in rising and falling contractile phases (mean tension at [illegible] 12% of peak tension)

[illegible] at peak measured from force records and those in rising and falling phases extrapolated from force–velocity [illegible]. k_{se} stiffness of elastic element, from eq. (5). a/P and b parameters of Hill eq. (2). Velocities measured 100 ms [illegible] phase. V_{max} extrapolated maximal shortening velocity. Significance levels based on paired Students' t-test for [illegible] of rising vs. falling phases. Mean values ±S.E. [illegible]=4

	P (mN/mm²)	k_{se}	a/P	b (lengths/s)	V_{max} (lengths/s)
[illegible]k	120±18	56.7±2.1	0.18±0.01	0.104±0.009	0.59±0.05
[illegible] phase	131±16	61.2± [illegible]	0.18±0.02	0.1 [illegible]1±0.007	0.69±0.06
	<0.01	<0.05	n.s.	<0.01	<0.01
[illegible] phase	93±15	55.2±3.5	0.16±0.01	0.078±0.003	0.49±0.04

[illegible]ce. Recordings at higher sweep rate (see e.g. Fig. [illegible] Johansson, Hellstrand & Uvelius 1978) show [illegible] the first large deflection in length and force is [illegible]pleted in 1[illegible] ms, that oscillations occurring [illegible] the largest load steps may last for 10–1[illegible] ms at [illegible], and that no obvious phase difference is seen [illegible]tween the oscillations in length and force. The



[illegible] 3. Plots obtained from computer sampling at 2 kHz of [illegible] changes which occurred in one preparation (initial [illegible]=13 mm, weight 2.1 mg) subjected to 6 different [illegible] steps at the peak of isometric contractions (13.0–15.5 [illegible]). The relative load is given by the number at each [illegible]. Length change after the initial recoil was analyzed [illegible] double exponential (see text). The fits are shown [illegible] figure as broken lines but can barely be distinguished [illegible] to overlap with tracings of data samples.

shortening of the muscle after the initial elastic recoil is characterized by a continuous decrease in velocity. It is clearly seen from Fig. 2A that this decrease in the rate of shortening is quite fast for about 50 ms and proceeds much slower thereafter.

Fig. [illegible]B shows 2 superimposed recordings from an experiment in which the preparation was subjected to an isotonic quick release from 16 mN to 5.6 mN elicited in the rising and in the falling phase of the isometric contraction. The fast sweeps of the force signal superimpose, showing that the load was practically identical over the first [illegible]50 ms in the two cases. The slow sweep shows a late rise in force particularly in the tracing from the early release, a deviation which is due to the inability of the lever spring to keep the load constant at the extreme degrees of shortening. The consecutive components of the length change described in connection with panel A can be identified also in these responses. It can be seen that the early recoil associated with the force step is nearly the same in the two superimposed responses. However, close inspection of the tracings reveal that the immediate shortening is somewhat larger in the release from the descent of the isometric contraction than in that from the ascent. The two responses follow a similar time course in the early part of the subsequent shortening but thereafter shortening is much slower in the response obtained during relaxation.

Fig. 3 is given to exemplify the kind of plots and data which are obtained by the computer analysis according to eq. 4. The figure shows 6 tracings of length change from an experiment in which different force steps were performed at the peak of the isometric contraction. The relative loads to which the muscle was released or stretched are given by the numbers at each tracing.

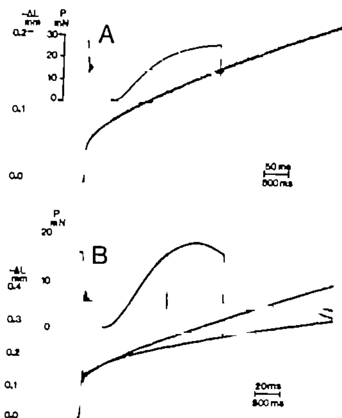


Fig 2 Oscilloscope recordings of force and change in length from expts on AC stimulated preparations of urinary bladder smooth muscle Slow sweeps show the time course of the isometric contractions and the load steps elicited by release of the isotonic lever Fast sweeps triggered by the release illustrate the force steps and the changes in length. The most rapid responses not clearly visible on the film have been indicated by dotted lines. The horizontal line in the middle of each panel is the zero force baseline

In *A* the muscle (initial length = 4 6 mm weight = 1 7 mg) was stimulated for 3 s and the release was triggered at the peak of the isometric contraction giving a force step from 7 to 16 mN The change in length is seen to consist of a rapid recoil and a subsequent shortening the velocity of which decreases rapidly within the first 50 ms and more slowly thereafter

B shows two superimposed recordings from a muscle (initial length = 6.5 mm weight = 1 2 mg) stimulated for 2 s Releases were elicited from an isometric force of 16 mN to 5 6 mN in the rising and in the falling part of the contraction respectively (slow sweeps) As shown by the fast sweeps the force steps were practically identical in the two responses Compared to the early release the length response obtained during relaxation showed a slightly greater elastic recoil similar rates of early shortening and lower rates of late shortening.

the contraction, the records obtained during relaxation showed a strong curvature due to declining activation and in these expts computation had therefore to be limited to the 250 ms interval i e. to the first 65 points formed as above. The starting point of the counting interval was chosen from inspection of a graphic representation of the sample values on the computer terminal. Computation was started 9–12 ms (in most cases 10 ms) after the trigger pulse which includes a delay of about 2 ms in the relay before any length change occurred. At that point the tension had stabilized at the new level and length oscillations had subsided.

The computation was carried out using the linearization (or Taylor series) method for non-linear regression as described by Draper & Smith (1966). The fitted curve was displayed graphically together with the sample values (cf. Fig. 3) The first intersection of the curve with the experimental record was defined as the end-point of the SE recoil" This point occurred 1 2 ms after the start of the tension change This point was also used for computation of the initial velocity V_{in} by differentiation of the fitted curve. Velocity values applying to other points in time were similarly found by differentiation.

Force–velocity relations were calculated in the basis of velocities at 100 ms after release This conforms to the earlier practice (Hellstrand & Johansson 1975). The velocity at 100 ms should be representative of the steady shortening phase as the velocity transient has essentially subsided by then (Johansson et al. 1978). The Hill equation (2) was fitted to the force–velocity values in a computer program which minimizes the sum of the squares of the perpendicular distances from each point to the curve Since errors are likely in both coordinates and since the curve changes its slope very drastically from the lightest to the heaviest loads this was considered the best procedure Only velocity values for $P<P$ (shortening) were used for computation, although stretch values, where such were obtained were also displayed by the plotter together with the extrapolated Hill curve

The SE recoils for the different load steps were fitted to a logarithmic curve.

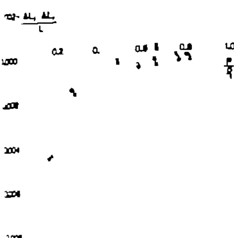
$$\frac{\Delta L}{L} = \frac{1}{k} \times \ln \frac{P-B}{A}$$

where A B and k are constants and L is the initial muscle length. The computation procedure was as for the force-velocity relation except that stretch values for the SE were included if obtained experimentally

RESULTS

1 *General description of the responses*

Fig 2 illustrates original oscilloscope recordings from experiments on urinary bladder smooth muscle The preparation in panel A developed a maximal isometric force of 27 mN as shown by the slow sweep tracing At this peak of the contraction a force step to 16 mN was performed The force step and the resulting changes in muscle length are shown by the fast sweep The shortening response of the muscle begins with an early rapid component which occurs simultaneously with the change in



[Fig.] 5. Differences in series elastic recoil between releases to comparable load steps elicited on the falling and the rising part of the contraction ($\Delta L_f - \Delta L_r$). The [figure] gives data from 4 expts. of this kind, each represented by a special symbol. Note that only in a few cases the recoil on the rising part exceed that on the falling [part].

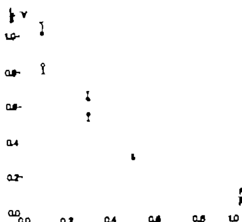
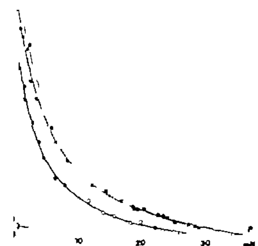


Fig. 7. Initial velocity of shortening (V) following the elastic recoil obtained in releases in the rising (filled circles) and falling (open circles) phase of the contraction. Data from 4 expts. were grouped according to P/P_0. Mean values and SEM are shown. Differences are statistically significant only in the two groups representing the lowest and the highest P/P_0.



[Fig.] 6. Velocity of shortening (V_s) 100 ms after release as function of force. The preparation (initial length = 3.4 [m]m, weight = 0.8 mg) was subjected to isotonic releases in [the] rising (filled circles) and falling (open circles) phase of [the] contraction, and at the peak (crosses). Force–velocity [curves] obtained by fitting the data to Hill's equation are [shown] by dotted, full and broken line, respectively.

property mainly of the transient isotonic response following the release (see below).

C. Shortening as a double exponential process
From the original recordings in Fig. 2 is seen that the late component of shortening, from about 50 ms after release and onwards, appears very different depending on whether release is made in the rising or falling phase of the contraction. Besides being slower after the late release, the shortening record is visibly more curved. The assumptions justifying an exponential fit (see Methods) to the late shortening component are not fulfilled for a release at falling isometric tension because of decaying activation of the muscle during that phase. Nevertheless the shortening records could be well fitted to exponential curves although the parameters obtained were dependent on the time interval over which the analysis was extended. This indicates that a more complicated model would be necessary for an adequate description of the entire time course of shortening during decaying activation. However for the limited purpose of separating the transient isotonic response from the later shortening phase the two-component exponential model was useful.

The parameters obtained for the fits to the late shortening component, amplitude (A_3) and time constant (τ_3) are shown grouped in Fig. 8. For the releases during rising tension they show the expected behaviour (eq. 3): A_3 decreases and τ_3 (in-

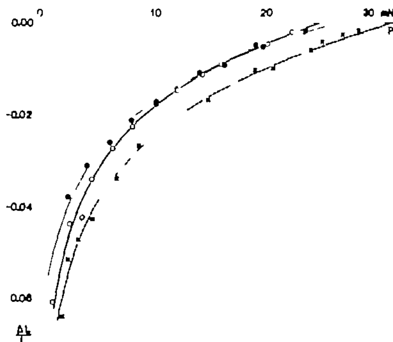


Fig 4 Series elastic recoils measured in one preparation (initial length=3.4 mm, weight=0 8 mg) subjected to isotonic releases from an isometric force of ?7 mN in the rising (filled circles) and falling (open circles) phase of the contraction and from the peak of the contractions where the mean force was 32 mN (crosses). Fits to logarithmic function (see text) are shown by dotted full and broken line respectively

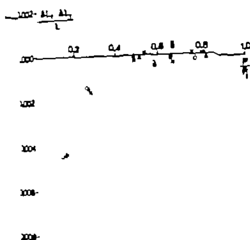
2 *Isotonic releases from a fixed tension level in ascending and descending phases of contraction*

This section describes results from expts of the kind illustrated in Fig 2B Releases to each afterload were made from the peak tension and from a fixed tension level P (average 82% of peak tension) in the rising and in the falling phase of contraction (in the latter case after cessation of stimulation)

A *Series elasticity* Fig 4 shows data from one expt. where the immediate length recoils after the release have been plotted against the afterload P It is seen that in general releases in the falling phase (open circles) caused numerically somewhat larger length changes than those in the rising phase (filled circles) The SE load-extension curve at the peak of contraction (crosses) is displaced downward with respect to the curves obtained with releases from the lower tension level Logarithmic curves (eq 5) were fitted to the data The values obtained for the parameter k characterizing the relation between stiffness and force are shown in Table 1 In the rising phase k is higher than in the falling phase Intermediate values are found at the peak A more direct illustration of the difference in SE recoil between the rising and the falling phase is given [in] Fig. 5

B *Shortening velocities* The shortening ve[loc]ities (V_1) measured 100 ms after release are p[lot]ted against P in Fig 6. From the fitting of Hill's e[q.] (2) to the results it is seen that both V_{max} (intercep[t] on velocity axis) and P (intercept on force axis) [are] greater in the rising than in the falling contract[ion] phase despite that the releases were made from the same force level At the peak of the contraction V_{max} is slightly lower than during the rising phase

The initial shortening velocity V measured im[]mediately after the SE recoil is shown in Fig. 7 Th[e] data from all 4 expts 47 releases in rising and [] falling contraction phase were grouped accordi[ng] to P/P_i into classes with a width of 0.2. In all class[es] V was greater in the releases during rising tension but the difference is significant for the highest [and] the lowest group only In contrast the differen[ces] in V are greater as shown e g. by a comparison [of] average values of V_1/V (approx. 2.3 at rising [and] 4 3 at falling tension) It should be emphasized [] that we do not regard the very high shortening ve[]locities measured initially as characteristic of [the] energy-dependent active shortening response [of] smooth muscle In[] consider [] [illegible]



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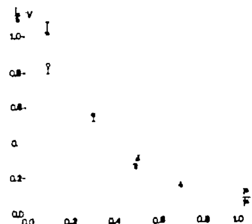


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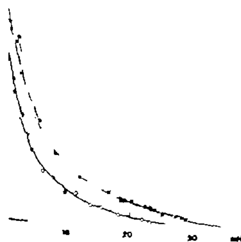


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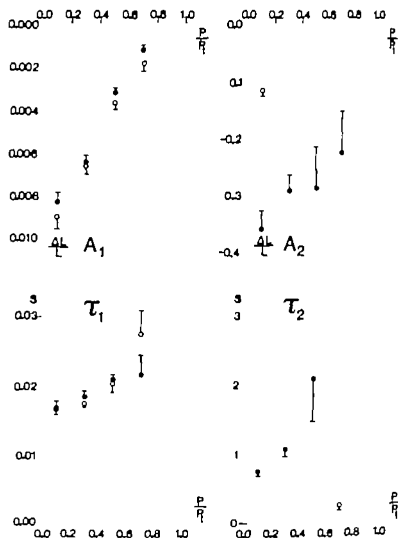


Fig 8 Amplitudes (A) and time constants (τ) for the early (1) and the late (2) component obtained by analyzing the shortening after the elastic recoil in terms of a double exponential model (see text) Symbols and grouping of data from 4 expts. as in Fig. 7

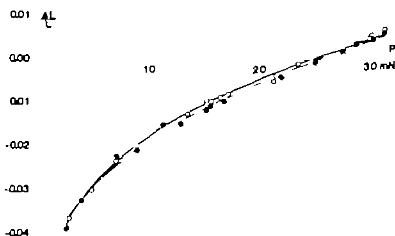
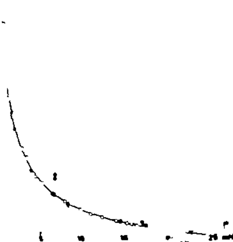


Fig 9 Series elastic responses measured in one preparation (initial length = 4 6 mm weight = 1 7 mg) subjected to releases and stretches t the peak of the isometric contraction at 37 (filled circles) and 27°C (open circles) The mean peak force in the different contractions was 27.3 and 26.. mN respectively Fitting of data to logarithmic function (see text) is shown by dotted and full lines, respectively



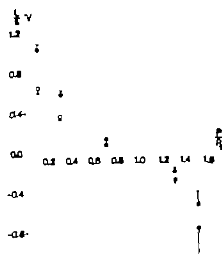


Fig. 11 Initial velocity of shortening (V_i) following the elastic recoil obtained in releases and stretches at the peak of the isometric contractions at 37 (filled circles) and 27°C (open circles). Data from 5 expts. grouped according to P/P. Mean values and S.E. are shown.

Fig. 10 Velocity of shortening (V_i) as a function of force after releases and stretches elicited at the peak of [illegible] contractions at 37 (filled circles) and 27°C (open circles). The mean peak force was 18.7 and 18.8 mN respectively in this preparation (initial length = 4.0 mm, [illegible] = 1.0 mg). Force–velocity curves obtained by fitting [illegible] below peak force to Hill equation are shown by [illegible] and full line.

[illegible] rate constant) increases as P/P increases. However, for the releases at falling tension, though A decreases, [illegible] tends to decrease for [illegible] P/P.

The small difference in V for releases during [rising] and falling tension (Fig. 7) and the appearance of the original records (Fig. [illegible]B) suggest that the parameters characterizing the exponential fit to the early shortening component should be similar. Fig. [illegible] shows the amplitudes (A) and the time constants of this component obtained from the exponential fits, grouped as before. Evidently the transient [early] response is very similar whether the release is made during the rising or during the falling phase of isometric tension. This result contrasts with the large differences noted in the late shortening component.

Effects of temperature

[In] 5 expts. releases from the peak of the isometric tension were performed both at 37°C and 27°C. In 4 of these expts. releases were also performed with afterloads greater than the isometric tension producing lengthening responses of the muscle instead of shortening. The term release here applies to both kinds of expts. Peak tensions in individual contractions within any experimental run varied by not more than ±10%. The influence of this variation on SE data was corrected for at each temperature separately.

A. *Series elasticity.* The SE characteristics at the two temperatures are shown for one expt. in Fig. 9. There is no consistent difference in the SE recoils measured for comparable force steps at the two temperatures. The stiffness values reported in Table [illegible] also show no significant variation in SE properties over this temperature range. As reported earlier (Johansson et al. 1978) the SE load-extension curves can be continued through the isometric point into the region of stretch responses.

B. *Shortening velocities.* Force-velocity relations at 100 ms after release at 37 and 27°C are exemplified by Fig. 10 and summarized in Table 2. Velocities of shortening at all relative loads are higher at 37°C with a mean V_{max} ratio of 1.36. It may be emphasized that the stimulus parameters were adjusted at the shift of temperature in these expts. in order to obtain peak forces of similar magnitude which was considered to make the comparisons of SE recoils and isotonic velocity transients more reliable. However these attempts to avoid temperature induced changes in P should be kept in

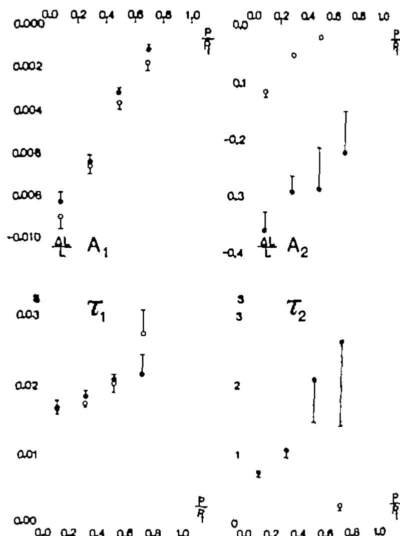


Fig 8 Amplitudes (*A*) and time constants (*τ*) for the early (1) and the late (2) component obtained by analyzing the shortening after the elastic recoil in terms of a double exponential model (see text) Symbols and grouping of data from 4 expts. as in Fig. 7

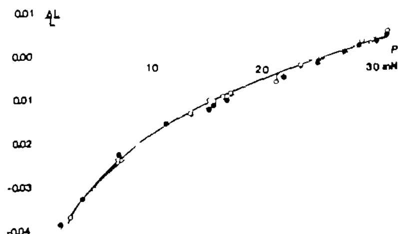


Fig 9 Series elastic responses measured in one preparation (initial length = 4 6 mm weight = 1 7 mg) subjected to releases and stretches t the peak of the isometric contraction at 37 (filled circles) and 27°C (open circles) The mean peak force in the different contractions was 27.3 and 26.2 mN respectively Fitting of data to logarithmic function (see text) is shown by dotted and full lines respectively

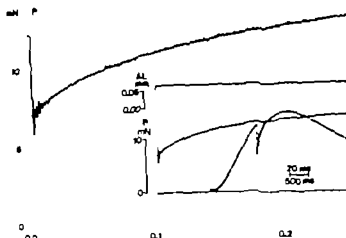


Fig. 13. Small inset figure shows direct oscilloscope recording of response to a length step in preparation (initial length = 5.9 mm) stimulated for 2.5 s. Slow sweep shows the isometric contractile force and the response to a length step given shortly before the peak of the contraction. The length step and the early changes in force are shown by the fast sweep. The rapid components not clearly visible on the film have been indicated by dotted lines. The large scale figure shows plot of the first 250 ms of the force tracing obtained by computer sampling as in Fig. 3. Recovery of force following the initial drop was analyzed as a double exponential (see text). The fitted curve is shown by the broken line. The parameters of the fit are: A = 1.4 mN, = 26 ms, A = 6.2 mN, 196 ms.

...y component are shown in Fig. 12. For $P/P < 1$ ...same general characteristics are seen as in the ...from releases at rising tension in Fig. 8. For >1 becomes very small. Inspection of the ...rds in Fig. 3 suggests that for the stretch re...nses the separation into early and late compo...ts is less clear than during shortening. The re...s of the double-exponential analysis was also ...nd to be very dependent on the time interval ...r which computation was extended. It is thus ...sible that the quantities measured as in the ...tch responses are actually much influenced by ...y early component. Thus A and values for >1 are not easily interpreted and are shown ...re for comparison only.

The temperature dependence is more pronounced in A than in but we would not like to ...ce much emphasis on the effects on these ...rameters. It is possible that also in the releases at ...t peak of the contraction there is some degree of ...activation influencing the late component in a ...milar way as discussed for the releases during de...ping isometric tension in connection with Fig. 8. In Fig. 12 are also shown the parameters of the ...ponential fit to the isotonic transient component, A and There is no consistent temperature dependence in A. More notable is the strong temperature dependence of τ in the three classes with the lowest P/P_0. For this range of loads the early component is most easily separated from the late component of the isotonic length response (cf. Fig. 3). For $P>P$ the temperature dependence in seems to be reversed. As noted above this may be a consequence of inadequate separation in the stretch responses of the early and late components.

4. Other force and velocity transients

Beside the isotonic velocity transients following step changes in force which we have analyzed above there are other mechanical transients to be observed in the smooth muscle. Without providing a systematic description we shall illustrate their general features as this may contribute to the subsequent discussion of the properties of the contractile system in the smooth muscle.

In Fig. 13 the inset illustrates an original oscilloscope tracing of a response to a step change in length, performed before the isometric contraction reached its maximum. The length step which

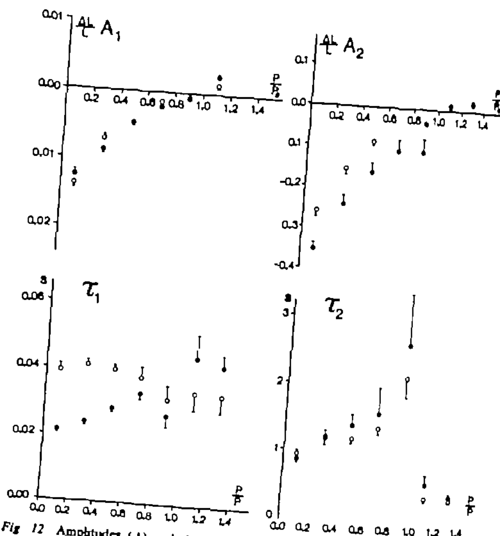


Fig 12 Amplitudes (A) and time constants (τ) for the early (1) and the late (2) component obtained by analyzing the length change after the elastic recoil in terms of a double exponential model (see text). Symbols and grouping of data from 5 expts. as in Fig. 11

mind when considering for instance the weak temperature dependence of V_{max} The values reported in Table 2 are accordingly not necessarily representative of the temperature effects under conditions of maximal contraction at both temperatures

In the expt shown in Fig 10 the force–velocity relation has been extended to the region where $P > P_0$ although only values for $P < P$ were used for fitting the curves As reported before (Hellstrand Uvelius & Johansson 1977 Johansson et al. 197[illegible] the lengthening velocities at 100 ms are greater th[illegible] an extrapolation of the Hill relation would indica[illegible]

The initial velocities V_1 are shown in Fig. 11 grouped from a total of 88 releases at 37°C and [illegible] 27°C The temperature effect on V_1 is similar to that on V as indicated by the average values of [illegible] (about 2 6 at 37°C and 2 3 at 27°C)

C *Shortening as a double exponential process* The parameters A_2 and τ_2 describing the late slow [illegible]

Table 2. *Mechanical parameters at 37°C and 27°C Release at peak of contraction*
Columns as in Table 1 Significance levels based on paired Student s *t*-test Mean values ±S.E $n=5$

	P (mN/mm²)	k_{sec}	a/P	b (lengths/s)	V_{max} (lengths/s)
37 °C	84±16	42.2±3.5	0.16±0.01	0.095±0 007	0 61±0 02
P	n.s	n.s	n s	<0.05	<0 01
27°C	78±14	40.8±3.5	0 16±0 01	0 075±0.010	0 46±0 04

…ate term, it is used here in the operational …e to designate the immediate elastic response to …p change in force.

…ork on skeletal muscle has revealed transient …onses to step changes in force or length which …e been accounted for within the framework of …sliding filament mechanism by different models …ross-bridge behaviour (Podolsky 1960 Civan & …olsky 1966, Podolsky Nolan & Zaveler 1969 …ley & Simmons 1971 1972, Podolsky & Nolan …, Julian, Sollins & Sollins 1972 Huxley 1974 …ott & Steiger 1977 Ford Huxley & Simmons …). In smooth muscle definite evidence for a …ing filament mechanism of contraction is still …ing, although thick and thin filaments, as well as …s-bridges, have been demonstrated (Ashton …lyo & Somlyo 1975 Somlyo 1978). In particu… it is not known how the filaments are mechani…y coupled to each other and how force is trans…ed between cells. In view of these difficulties it …erhaps prudent to limit discussion of smooth …cle mechanics to purely phenomenological …dels with no reference to underlying molecular …sms. However precisely because we lack the …nt structural knowledge it should be justified …ke the alternative approach and see to what …nt the concepts of skeletal muscle contraction …y be applied to smooth muscle. We shall thus in …following take as a basis for part of our con…rations the assumption that smooth muscle con…ction occurs via cyclic operation of cross-bridges …h properties similar to those of skeletal muscle …l that a contractile unit analogous to the sarco…re may be identified in the smooth muscle Since …dimensions *of this sarcomere analogue is not* …own, comparisons between muscle types have to …performed on the basis of muscle lengths.

The viscoelastic behaviour of the smooth muscle …y possibly arise from passive structures in the …scle, not directly involved in contraction. Re…se experiments performed on relaxed muscles at …l actual preload (<0.3 mN) produces an elastic …oil and a following viscous adjustment to a new …t length (unpublished results) Due to the small …ces involved, this passive viscosity will however …ence the active responses only negligibly and … may conclude that the major part of the vis…elasticity of the active muscle is a property of the …tractile state as such (cf. Mulvany 1979) It …ould be noted that a possible source of viscosity …the relaxed muscle is the contractile apparatus itself since the presence of attached cross-bridges in relaxed smooth muscle has been proposed (Siegman et al. 1976).

In the following, the three phases of the length response mentioned above will be discussed separately beginning with those related *to the two elements* of the classical muscle model

1 *Elastic recoil*

The elastic recoils observed in the present study when releasing the muscle from its maximal active force to a minimal load corresponded to 4–6% of *the initial muscle length*. The overall SE stiffness was somewhat smaller (Tables 1 and …) than in the expts. reported earlier (Uvelius 1977 Uvelius, Hellstrand & Johansson 1977 Johansson et al 1978) Many factors such as animal age dissection procedure, initial muscle length etc. may influence the absolute magnitude of the SE recoil.

There is much to indicate that a major proportion of the elastic recoil in the smooth muscle occurs in passive structures which are indeed placed in series with the contractile units. The lack of appreciable *influence of temperature (Fig. 9) seems to support* the essentially passive nature of the elastic response in urinary bladder Also the close similarity of the SE recoils with corresponding force steps at different stages of the contraction, emphasized in our earlier study is consistent with this notion. However the detailed analysis performed in the present study revealed a small but systematic difference between the elastic recoils in the rising and falling phases of the contraction (Fig. 4 and 5 Table 1) This indicates very strongly that some fraction of *the elasticity resides in elements whose stiffness is* not exclusively force- but also time-dependent.

It may be pointed out that differences in SE between different stages of the isometric response have been observed earlier in other muscles. For instance Jewell & Wilkie (1958) found in controlled release expts. on frog sartorius that the series elastic component does become slightly more compliant during relaxation and Meiss & Sonnenblick (1974) have reported similar observations on cardiac muscle. In contrast, a recent study on rabbit *mesotubarium smooth muscle by Meiss (1978) indi*cated that muscle stiffness at all tensions was significantly increased during relaxation. Stiffness was determined in the latter study from the amplitude of the tension changes elicited by small sinusoidal length perturbations amounting to 0.5% of the mus-

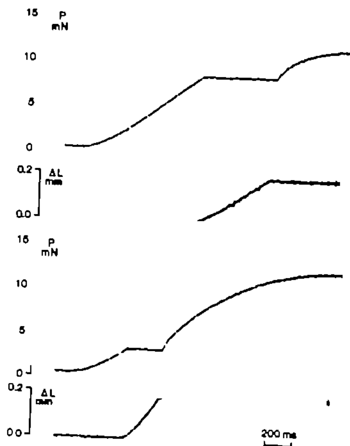


Fig 14 Oscilloscope recordings of force and change in length from a preparation (initial length = 4 6 mm) in which the isometric contraction was *interrupted* by a period of isotonic shortening against a load of 8.5 mN (above) and 3.3 mN (below) Note the gradual increase in dL/dt after the shift to isotonic conditions and the gradual decrease in dP/dt after return to isometric contraction

amounted to 1 1% of the original muscle length was associated with a simultaneous drop in force followed by force recovery Oscillations in the transducer disturb the recording over the first few ms after completion of the length step In order to characterize the time course of the subsequent force recovery a double exponential fit was made as for length changes above The large scale figure in Fig 13 shows a plot of the first 250 ms of the force record and the fitted curve (broken line) The time constant of the two components was τ =26 ms and τ_2=196 ms respectively Thus it was found by analysis of this and other responses to different length steps that force recovery in the bladder smooth muscle can be separated into two phases Fig. 13 was obtained in an experiment at 37°C and it should be noted that τ_1 is of about the same magnitude in this force recovery as in the length responses at this temperature shown in Fig 8 and 1? On the other hand the force recovery shows an appreciably lower τ_2 than that found in the analysis of length changes. This smaller difference between the time constants in the force responses explains why the break point is not as obvious in these recordings as in the length tracings of Fig. 2 and 3.

Fig 14 shows recordings from an expt in which the increase in isometric force during contraction was interrupted by a short period of isotonic shortening It can be seen that the muscle, in shifting from isometric to isotonic contraction, does not immediately assume its maximal velocity of shortening against the actual load but approaches this velocity gradually over some 200 ms. Conversely, when the muscle returns to isometric contraction the initial rate of rise in the transient is significantly higher than the dP/dt of the isometric contraction at the actual force level before the isotonic shortening Comparing the force transient of the lower panel with the isometric increase in force in the upper panel at corresponding times also shows a relatively higher dP/dt in the transient. A comparison representing both corresponding times and equal muscle lengths is obtained by looking at the transient of the upper and the late isometric contraction of the lower panel Again the rate of rise of tension in the transient is strikingly high. The recordings in Fig 14 thus demonstrate that the smooth muscle when shifting from isometric to isotonic contration or vice versa displays mechanical transients lasting up to 200 ms before new steady state situations are reached

DISCUSSION

The length response observed in the smooth muscle after a force step can be resolved into three parts (1) elastic recoil (2) rapid length change (3) steady length change The different nature of phases (2) and (3) has been demonstrated by superposition of two shortening records after release to the same afterload but from slightly different initial lengths (Johansson Hellstrand & Uvelius 1978). Phases (1) and (3) show properties that can be ascribed to a muscle model with an elastic and a contractile component coupled in series (Hill 1938) The transitional phase (2) is however not explained by this model in its original form It was also found in the present study that phase (1) is not altogether due to passive elastic elements, since one part of the elastic recoil depends on the level of activation (Fig. 4 and 9. Although series elas[illegible] is thus not a simple

cle length and having a frequency of 80 Hz From the present study smooth muscle appears stiffer during the increase in isometric force than during relaxation when elasticity is studied by rapid re leases

2 *Force–velocity relation*

The ability of the contractile element to shorten against different loads under isotonic conditions is summarized by the force–velocity relation It was demonstrated in our earlier study that such shortening velocities after quick releases should not be measured earlier than 100 ms after the release in order to represent a fully adapted contractile sys tem Absolute V_{max} values are considerably higher in the present study This may partly be attributed to the facts that the muscles were now mounted at a greater relative length developed greater active tensions and were stimulated electrically instead of by K When Hill s (1938) hyperbolic equation was fitted to results from releases at the peak of the isometric contraction curves with intercepts on the force axis somewhat above the real peak were obtained (e g Fig 6 and 10) The reason for this often seemed to be a slight upward convexity of the ex perimental curve for shortening velocities at high after loads Such deviation from the hyperbolic configuration has been described and analyzed in a study on skeletal muscle by Edman Mulieri & Scubon Mulieri (1976)

The dependence of the force–velocity curve on time relative to stimulation is illustrated by Fig 6 It can be seen that the curves obtained in the rising part of the contraction and at the peak show quite similar values of both P_0 and V_{max} suggesting that the AC stimulation causes an activation of the con tractile system which is quite constant over this period of time In an earlier study of spontaneous phasic contractions in portal vein (Hellstrand & Johansson 1975) the V_{max} values at the peak of the isometric responses were lower than during the rise in tension indicating that under the conditions of those experiments partial inactivation had occurred already at the time of the peak The extrapolated V_{max} and P_0 values of force–velocity curves obtained during declining isometric tension were low er than corresponding values in earlier stages of the contraction both in the previous study of portal vein and in the present study (Fig 6) If deactivation during relaxation is attributed to decreasing $[Ca^{2+}]_i$ the force–velocity results suggest that this ion may control not only the number of active con tractile sites determining isometric force but also molecular events determining the maximal velocity of shortening. This suggestion contrasts with the notion that Ca^{2+} would exert only a "polytropic" but not a "tachytropic" action on the contractile system of smooth muscle (Peiper 1977). The possibility that shortening velocity in smooth muscle is limited by "internal load" for instance due to rigor like linkages has not been excluded.

The effects of temperature on the force–velocity characteristics are illustrated by Fig. 10 and Table 2 As pointed out above it is important to note that stimulus parameters were adjusted when shifting from 27 to 37°C in order to obtain about equal peak force in the isometric responses The influence of temperature on force velocity parameters, such as V_{max} and the constants a/P_0 and b must be considered in the light of this experimental "standardization" of P_0 The results show that V_{max} at 37° exceeds that at 27° by an average of 36% which implies that shortening velocity is clearly more temperature dependent than force generation (cf Peiper Laven & Ehl 1975 Stephens Cardinal & Simmons 1977) It is conceivable that an increase in P_0 and a further increase of V_{max} would have occurred with maximal stimulation at 37° Systematic determinations of Q_{10} values were however not attempted in this study

The characteristics of the force–velocity curve at forces $>P_0$ should be revealed by the isotonic stretches of the contracted muscle A deviation of the data points towards greater lengthening velocities than corresponding to an extrapolation of Hill's hyperbola is indicated by Fig 10 and by our earlier studies (Hellstrand Uvelius & Johansson 1977 Johansson et al 1978) However it is uncertain whether in the stretches the contractile system has really adjusted to the new load at 100 ms after release (see below)

3 *Velocity transients*

The double exponential fit to the length response after the elastic recoil is of course not the only possible mode of analysis However since other evidence suggests that two components of approximately exponential shape are present in the responses (Johansson Hellstrand & Uvelius 1978) it was considered that the double-exponential model would provide a fair description of the amplitudes and time courses o[illegible] The large difference in

me constants observed for the two compo-
n the shortening responses (Fig. 8 and 12)
ss that the separation into two components
e made with good accuracy. In the case of the
ement responses, however, the separation
wo components is less clear. There is a possi-
that the late component is actually a rather
steady lengthening, so that a considerable por-
f the curved length record seen during the 500
f analysis is actually a part of the transient
nse (cf. Fig. 3).

ansient effects rather similar to those con-
ed by us have recently been reported by Mul-
(1978, 1979) in smooth muscle from small ar
l resistance vessels. These responses, lasting
t 150 ms, were characterized by an initial ve-
y about 6 times higher than that of the eventual
ly shortening. For a comparison it should be
d that the shortening responses in general seem
slower in the arterial muscle than in the blad-

he question arises whether the transient re-
nses found in the smooth muscle may be of a
lar nature as those in skeletal muscle, although
appearance and the time scale are very differ-
The amplitude (A_1) of the length transient in
expts. cannot readily be compared either with
displacement deviations measured by Civan
Podolsky (1966) or with the length steps
loyed in studies of force transients (e.g. Huxley
immons 1971, Abbott & Steiger 1977). We want
xplore the possibility of interpreting the tran-
ts in the smooth muscle in terms of events within
ched cross-bridges in line with the model of
xley & Simmons (1971). An upper bound on the
plitude of the length transient per half sarcomere
logue in such a model would thus be the range
r which an individual cross-bridge is able to
rate. In skeletal muscle this range appears to be
und 12 nm (Huxley 1974), corresponding to 1–
% of the half sarcomere length. The amplitude
(Fig. 8 and 12) found in our expts. is of about this
gnitude when related to muscle length. The size
the isotonic length transient in the smooth muscle
thus consistent with the idea that the phenome-
n relates to cross-bridge movements and is
alogous to the transient events observed in
eletal muscle.

If the interpretation of the mechanical transients
the smooth muscle in cross-bridge terms is ac-
pted, then the time course of the transients
should relate to events within the cross-bridge cy-
cle. The time constants of the isotonic transients for
shortening at 37°C lie in the range of 15 to 30 ms
(Fig. 8 and 12) implying a 95% decay in 45–90 ms.
The isotonic transients observed in skeletal muscle
at 0–2°C (e.g. Civan & Podolsky 1966, Huxley
1971) are divided into 4 phases which correspond
to similar phases in the tension transients (e.g. Hux-
ley 1974). After the elastic recoil (phase 1) a period
of rapid shortening lasting a few ms (phase 2) is
seen, followed by a slowing down or reversal of
shortening for the next 5–20 ms (phase 3). Some
damped oscillations may sometimes precede the
steady shortening (phase 4). Phase 2 is attributed to
conformational changes in attached cross-bridges,
whereas phase 3 would result from a change in the
balance between attachment and detachment of the
cross-bridges, detachment being the faster process.
With regard to phase 3, Huxley (1971) has also
suggested that "after a side-piece has detached from
one thin filament, it needs to undergo one or more
reactions before it is able to attach afresh, or at least
before it can exert tension". Estimates of the total
duration of the cross-bridge cycle from energetic
studies of skeletal muscle at 0°C have given values
of 0.34 s in isometric contraction and 0.12 s during
shortening with maximal energy turnover (Curtin et
al. 1974). It thus seems that the cross bridges will
remain in the tension producing state for only a
small fraction of their cycle time. In pig carotid
artery at 37°C the cross-bridge cycle time in isomet-
ric contraction has been estimated by a similar ap-
proach to be about 0.75 s (Paul, Glück & Rüegg
1976).

In the mechanical transients observed in the
smooth muscle, phases "2" and "3" as defined for
skeletal muscle above cannot be distinguished from
each other. Possibly all of the early exponential
response should be compared to phase 2 of skeletal
muscle, but in that case its time course will be an
order of magnitude slower. It is also possible, how-
ever, that since the transient in the bladder muscle
smoothly merges into the steady shortening or ten-
sion recovery, it actually represents a composite of
the events occurring during phases 2 and 3 in
skeletal muscle. Its duration should thus be com-
pared to the total duration of 10–25 ms for phases
2+3 in the skeletal muscle. This may not be un-
reasonable since the V_{max} of the skeletal muscle at
?°C (e.g. Edman, Mulieri & Scubon-Mulieri 1976) is
at least 3 times that of the bladder muscle at 37°C. It

cle length and having a frequency of 80 Hz From the present study smooth muscle appears stiffer during the increase in isometric force than during relaxation when elasticity is studied by rapid re leases

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is interesting that a slowly contracting skeletal muscle of the tortoise shows a protracted force transient following a length step (Heinl Kuhn & Rüegg 1974) Similar to the transients in skeletal muscle those of the smooth muscle have a slower time course the smaller the force or length step This dependence is however not as strong in the smooth muscle With moderate and large force steps at which the resolution of the isotonic transient is most clear-cut its time constant is markedly temperature dependent (τ_1 in Fig 12) By contrast there is little influence of the degree of activation as indicated by the comparison of the transients in the contraction and relaxation phase (τ_1 in Fig 8) This may indicate that the possible effect of Ca^{2+} on cross bridge dynamics (cf above) is confined to steps in the cycle other than those responsible for the transient

Conformational changes in attached cross-bridges and adjustment of their rates of detachment and attachment to the new mechanical constraints after a force step may thus not be as separate in time in smooth muscle as they appear to be in skeletal muscle In relation to this suggestion the kinetic study by Marston & Taylor (1978) of actomyosin from chicken gizzard smooth muscle is of interest They showed that the comparatively low ATPase activity is explained by a rate-limiting step involving a long-lived attached actin-myosin complex It is possible that the amount of time spent by the cross-bridges in the attached state is considerably greater in smooth than in striated muscle This may also explain the low energetic tension cost in the smooth muscle

4 *Velocity step experiments*

The gradual adjustments of dL/dt and dP/dt on transition from isometric to isotonic conditions and vice versa as illustrated in Fig. 14 have a longer duration than the transient responses to a step change in length or force In some cases we have actually observed that a small force step in the rising contraction phase is followed by both kinds of "transient" effects resulting in a sigmoidal shortening curve Since force is constant during shortening there is nothing to indicate that the acceleration on isometric–isotonic transition would be due to friction within the apparatus It is also difficult to imagine a mechanical artifact that would produce the transient increase in dP/dt on isotonic–isometric transition

In terms of kinetic models which imply fewer attached cross-bridges during shortening than during isometric contraction these effects may possibly arise from the changes in the rates of attachment and detachment of bridges which will be the result of the change in relative filament velocity Good... Mates (1975) have analysed the consequences of cross-bridge compliance on the response of A... Huxley's (1957) muscle model to changes in mechanical constraints It was found that on isometric–isotonic transition relative filament velocity should suddenly increase and vice versa This analysis and that of Julian & Sollins (19... also predicts the differences in stiffness during contraction and relaxation discussed above.

The present study has shown that it is possible to achieve a characterization of the mechanical behaviour of a smooth muscle preparation which goes beyond the elements of the classical muscle model The time-dependent properties of the elastic recoil speak against a purely passive SE and may be compatible with a cross-bridge theory of contraction The transient length response following the elastic recoil may be dominated by a change in the distribution of conformations of bridges in the attached position

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a Physiol Scand 1979 106, 239–40

bservations on the localization of carbonic hydrase in muscle

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ynihan (1977) has found carbonic anhydrase ac ty in rat skeletal muscle M soleus (which is dominantly red) contained a significantly higher centration of the enzyme than the predominant white m. extensor digitorum longus and m. vastus lateralis. It appears of interest to examine by a tochemical method the localization of the enzyme in the muscle and also to find out if there are ferences in enzyme localization between red and ite muscle fibers

all pieces of m. soleus and m. vastus lateralis from rat re fixed by immersion in glutaraldehyde (2 % buffered h phosphate) and embedded in JB-4 plastic, sectioned d incubated for demonstration of carbonic anhydrase ivity according to the modified Hansson method de ibed by Ridderstråle (1976)

e description given refers to what appears to be pical white fibers in m vastus and to red fibers in soleus. In general the pattern of staining is simi r in the two muscles.

The strongest staining is found in the endothelium of the capillaries (Figs 1 2) The sarcolemma or its immediate neighbourhood is clearly ained. With equal incubation time and section ickness the staining is usually heavier in m. vastus teralis than in m soleus The membrane-associated staining shows regular indentations (Fig.) Faint diffuse staining appears all over the muscle bers.

In two respects the staining pattern differs in the two muscles. Nuclei are stained in m. soleus In m. vastus lateralis the connective tissue is stained Acetazolamide in the concentration used (10 μM) inhibits all staining except weak diffuse staining in some muscle fibers and nuclei.

Zborowska-Sluis et al. (1974) found indirect evidence for the presence of carbonic anhydrase in skeletal muscle Moynihan (1977) determined the concentration of carbonic anhydrase in different muscles from rat. He found that m soleus m vastus lateralis and m extensor digitorum longus had significant concentrations of the enzyme after correction for the enzyme content in the blood M soleus with mainly red fibers had a significantly higher concentration than the other two with predominantly white fibers. Lönnerholm (1974) shows a figure of dogfish muscle showing no staining of the muscle fibers but a capillary blackened by the carbonic anhydrase staining reaction

The present study shows the strongest staining to be localized to the endothelium of the capillaries. Weaker staining was found at the sarcolemma M soleus is more richly supplied with capillaries than m. vastus lateralis and this could contribute to the higher concentration of carbonic anhydrase found by Moynihan in m soleus.

It is impossible (with the light microscope) to ascertain if the superficial staining of the fibers is

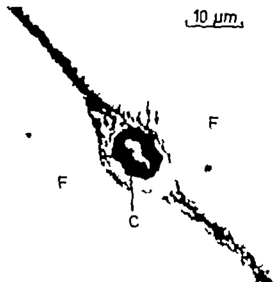


Fig 1 M vastus lateralis. Space between two fibers (F) with capillary (C). Arrows point to membrane indentations

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...ctromechanical delay in skeletal muscle ...der normal movement conditions

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NORMAN R. W. & KOMI P V Electromechanical delay in skeletal muscle under normal movement conditions. Acta Physiol Scand 1979 106. 41-248. Received 10 Aug. 1978. ISSN 0001-6772. Kinesiology Laboratory Department of Biology of Physical Activity University of Jyväskylä, Finland.

Electromechanical delays (EMD), the time from onset of EMG activity to change in acceleration or deceleration of the forearm, were studied in concentric and eccentric contractions of biceps and triceps brachii muscles. Horizontal flexion and extension movements were performed at varying speeds by 10 subjects. EMD time in concentric contractions for biceps was 41±13 ms and for triceps was 36±11 ms and was not influenced by the velocity of the movement. In eccentric contractions at the slower velocity the biceps EMD time was 38±13 ms and shortened to 28±10 ms at the faster velocity The eccentric triceps EMD however was not significantly altered by movement velocity and averaged 30±7 ms. The data provided support for the hypothesis that stretching of the series elastic component to point where muscle force can be detected, is the primary determinant of the EMD phenomenon. However there are complex interactions of the effects on EMD of muscle fiber type composition whether the contraction is concentric or eccentric and the velocity of the movement as well as possible gamma ystem influence These complications require that consideration of electromechanical delay be made when phasic relationships between muscle force or joint torque generation from different muscles are inferred from EMGs.

...al investigators have commented on the im... ...nce of appreciation of the time lags between ... of electrical activity and tension in muscle ...tromechanical delay EMD) They state that ...appreciation is essential *to proper understand*... ...f the type of commands required of the cen... ...nervous system for the execution of different ...ments the roles of muscles in human move... ... and the apparent anomalies between EMG ...ity and body segment motion (cf. Grillner 1972, ...on et al 1976 Corser 1974) The use of ...romyograms as transducers of muscle force demands knowledge of electromechanical de...lays as well as the durations of persistence of ...cle force after EMG activity has ceased to ...ate selection of appropriate time constants for ...G filters (cf. Hof & van den Berg 1978)

...ecently Komi & Cavanagh (1977) have at...pted to study the meachanisms of EMD and ob...ed that the delay was shorter during eccentric than concentric contractions. They presented evidence which suggested that this difference was re lated to differences in the rate of change of length of the series elastic element of the muscle

In their experiment the forearm was passively moved back and forth by an external force prior to voluntary maximal muscular contraction. In the concentric condition the shortening of the series elastic element had to "catch up" to and exceed the passive velocity of shortening of the muscle before the transducer detected any force change. The passively lengthening muscle on the other hand added to the velocity of stretch of the series elastic component. Thus the detectable force on the force length curve of the series elastic element was reached sooner in the eccentric than in the concentric contraction

From this work one can hypothesize that any factor which shortens the time of reaching this de tectable force level of the series elastic component

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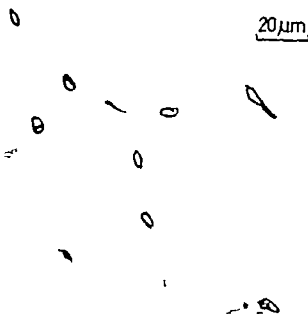


Fig 2 M soleus Numerous stained capillaries surrounding the muscle fibers

bound to the sarcolemma or located outside or inside the membrane The indentations of the staining into the muscle fibers at regular intervals may suggest that the enzyme is very closely associated with the membrane and follows it at least some distance along the transverse tubular system (T system)

Speculation with regard to the role of the carbonic anhydrase in muscle may be premature However Zborowska Sluis et al suggested facilitation of carbon dioxide transport according to the scheme proposed by Enns (1967) Several other functions of carbonic anhydrase in muscle appear possible

At least in some locations the enzyme may be involved in hydrogen ion transport

The production of lactic acid in the muscle fibers during anaerobic work must lead to formation of carbonic acid Rapid dehydration of this acid would diminish the resulting acidification

The carbon dioxide—bicarbonate system is a rapidly functioning buffer system only when carbonic anhydrase is available This may be of importance for the damping of rapid acid-base changes e.g during rhythmic contractions

Carbon dioxide formed during periods of capillary occlusion will mainly accumulate as bicarbonate (even without carbonic anhydrase) The bicarbonate may be more rapidly removed during the intermittent perfusion of the capillaries when the transformation of bicarbonate into carbon dioxide is catalyzed

However when speculating about the function of carbonic anhydrase in muscle it may be appropriate to point out that carbonic anhydrase is found in the walls of capillaries in many organs e.g. mucosa of duodenum cecum and colon in rabbits, stomach gland cecum and colon in rats, rumen of the goat, the avian shell gland (unpublished observations by the author) where processes connected with contractility are probably not important

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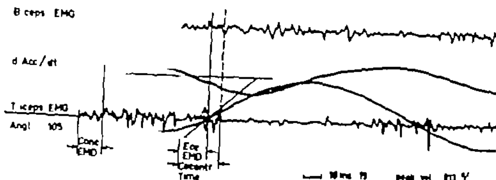


Fig 1 Schematic diagram of typical extension flexion movement *dAcc/dt* is rate of change of acceleration from piezoelectric accelerometer *Angle* is displacement curve from potentiometer Concentric (CONC) and eccentric contraction electromechanical delays (*EMD*) and biceps/triceps EMG coactivation (*Coactiv Time*) are identified at point of inflection (*A*) marks peak angular velocity and onset of deceleration of forearm extension.

should lead to a shortened EMD A number of such factors conceivably interact in normal body segment movements (1) Fast movements are considered by some to call on motor programs which activate fast motor units and their respective muscles (cf Hannerz 1974) These motor units have fast force rise times (Gydikov et al 1976) Thus fast movements whether produced by eccentric or concentric contractions should have shorter EMDs than slow movements (2) Muscle comprised of a preponderance of fast twitch fibers should yield shorter EMDs (3) In eccentric contractions there is the possibility of stretch reflexes augmenting activation of the α motor neuron pool This should result in shorter EMDs (4) Rack & Westbury (1969) have alluded to the possibility of increased muscle stiffness because of possible compression and consequent restriction of fluid flow in the transverse tubules Although Bahler (1967) has reported that the series elastic component is only lightly damped (about 300 dynes/cm/s for viscous resistance of rat muscle) Hatze s (1975) damping coefficient calculations which have been used in a successful simulation of a kicking movement imply appreciable visco-elastic resistance particularly at high angular velocities of the limb Indeed account of viscous damping of muscular contraction is taken in most current muscle and muscle/limb models (cf Bawa et al 1976 Glanz 1977 Hatze 1976) The presence of viscosity whatever the site should shorten electromechanical delay times in faster movements since the viscosity would stiffen the muscle /tendon/ bone linkage as a function of rate of change of length

The present study was an extension of Komi & Cavanagh s (1977) investigation of electromechanical delays to forearm movements at different velocities initiated by the subject from rest decelerated and reversed voluntarily This movement was somewhat more natural than that used in the previous study and contained concentric and eccentric contraction phases in the same trial. An attempt was made to test the hypothesis that rate of change of length of the series elastic component of the muscle was a major contributor to variations in EMD time

METHODS AND MATERIAL

Procedures

10 subjects 5 males and 5 females from 21 to 35 years of age (average 4 6) who had no history of neuropathy or myopathy participated in the experiment. They performed a series of horizontal forearm flexion-extensions and extension-flexions at 7 different angular velocities over two ranges of motion 28 trials in all. The peak angular velocities of the movements ranged from approximately 4 4 to 16.6 r/s, the fastest being about ... of reported peak angular velocities of similar movements and moments of inertia (cf Pertuzon & Bouisset ... Stothart 1973) Since some control of the range of velocities was desired the 5 slower movements were paced by the beat of a metronome The upper two velocities were near maximum and maximum efforts The extension-flexions began either at a 105° elbow angle and proceeded to 165° (180°=full extension) where the subject reversed the movement returned the forearm to a ... angle and stopped or from 70° to 135° to 70° The flexion-extensions began at either 165° or 135° joint angle The intent of the two ranges of motion was to determine whether there were muscle length effects on electromechanical delays

1 *Means (standard deviations) of EMD tabulated with respect to type of contraction of motion and peak angular velocity of*

Vel, o/s	EMD, ms	Vel, o/s	EMD, ms
concentric			
FE 165° 105°		FE 135° 70°	
504 (29 4)	44 (15.6)	520 (36 4)	37 (18 0)
778 (99 0)	39 (8 0)	858 (94 6)	42 (8.2)
concentric			
EF 70° 135°		EF 105° 165°	
518 (57 0)	23 (12.7)	406 (42.5)	29 (11 9)
828 (47.8)	22 (6 0)	823 (46 1)	30 (9.5)
eccentric			
EF 105–165°		EF 70°–135°	
406 (42.5)	35 (9.3)	518 (52 0)	41 (16.5)
823 (46 1)	27 (5.6)	828 (47.8)	29 (14.6)
eccentric			
FE 135° 70°		FE 165°–105°	
520 (36 4)	33 (6 9)	504 (29 4)	30 (6.8)
858 (94 6)	26 (3 7)	778 (99 0)	31 (8.5)

165° 105° is flexion/extension movement starting elbow angle of 165° (180° full extension), flexing to and immediately returning to 165°
70°–135° is an extension/flexion movement over the indicated and explained in note[a]

ch movement, which started and finished at rest, ined an initial concentric muscle contraction NC) to accelerate the forearm, an eccentric contrac (ECC) to decelerate and momentarily stop it, chang- CONC to accelerate the forearm in the opposite ion and finally another ECC to decelerate and stop movement. Only the initial CONC and ECC contrac were analyzed. Thus, in the extension-flexion move triceps contracted initially concentrically biceps ec rically in the flexion-extension the roles were re- ed.

Subjects and data structure

ects sat with their forearm and upper arm in horizontal position, the upper arm, perpendicular to the line the shoulders. The forearm was strapped to de which could rotate in horizontal plane around an on low friction bearings. The axis of rotating of elbow joint was aligned with that of the apparatus linear potentiometer attached to the axle, and piezoelectric accelerometer mounted tangentially to the aratus, permitted recording of angular displacement rate of change of acceleration. Electromyograms were orded with surface electrodes placed 2 cm apart over bellies of biceps and triceps brachii muscles. The EMGs were conditioned in Brookdeal differential amplifiers (Type 4432) at gain of 60 dB and bandwidth of 10-1000 Hz. These signals and 1 ms timing pulse were recorded at 30 ips on 5 FM channels of a Philips Analog 7 tape recorder and played back at 15 ips on Honeywell Visicorder Type 2106 oscillograph operated at 20 cm/s. The bandwidths of the tape recorder and oscillograph were sufficiently wide to avoid signal distortion.

The CONC EMD time was measured from the first sign of EMG activity to the onset of acceleration of the forearm. The ECC EMD then was from the onset of antagonist muscle EMG to the initiation of deceleration of the forearm. Onset of forearm deceleration coincided with the first point of inflection in the displacement curve. This point also was aligned, in most trials, with a point of inflection in the rate of change of acceleration curve. The two inflection points mutually confirmed the onset of deceleration. The peak instantaneous angular velocity was measured from the maximum slope in the displacement curve and was used to categorize the movement effort. Fig 1 is schematic representation of an experimental record with the electromechanical delays indicated. ECC delays were measured to resolution of approximately ±2.4 ms and CONC delays to approximately ±0 6 ms.

RESULTS

The electromechanical delay results are summarized in Table 1 with respect to range, direction of motion and starting position (FE or EF) the type of muscle contraction (concentric, eccentric) the primary muscle involved in the acceleration or deceleration of the forearm (biceps, triceps) and the peak angular velocity of the forearm in the first half of the movement (Vel)

The peak angular velocity measure was intended to reflect the effort involved in the movement rather than to represent a variable associated directly with the linear velocity of the series elastic component of the muscle (SEC), particularly in the concentric condition, in which the muscle started at zero velocity The simple movements studied however are probably largely preprogrammed (Melville Jones & Watt 1971) therefore a call for a fast movement would involve a faster stretching of the SEC than a call for a considerably slower movement. In ECC condition the peak angular velocity is proportional to the linear velocity of lengthening of the muscle at the onset of deceleration of the forearm (Pertuzon & Bouisset 1971) the instant which marked the end of the EMD This velocity measure is, therefore also quite closely related to the velocity of muscle lengthening at the onset of ECC EMG activity the beginning of the EMD some 25 to 40 ms earlier

The data were analyzed using an independent group factorial design analysis of variance The

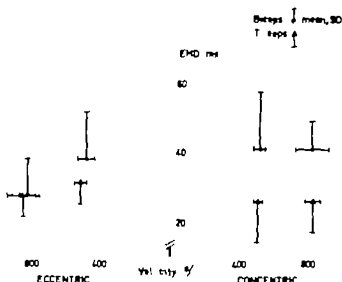


Fig 2 Electromechanical delay (*EMD*) plotted as function of peak forearm angular velocity for biceps and triceps brachii muscles in concentric and eccentric contractions Data were pooled over two ranges of motion (see text) Each data point represents 18 19 or 20 scores

reason for this type of analysis was that the data were organized to minimize variability in velocity of the movement by ranking the scores of the ten subjects according to forearm angular velocity then selecting ten of these scores for each experimental condition This meant that in some conditions the scores of some subjects were not represented This procedure thus had elements of both a repeated measure experimental design and an independent group design The latter more conservative statistical design was tested

The analysis of variance revealed statistically significant main effects differences in EMD times which could be attributed to velocity of movement the muscle involved and whether the contraction was CONC or ECC There were also significant interactions among these variables There was no significant main effect of "range of motion and starting position a variable that was intended to study possible effects of muscle length on EMD nor did this variable interact with any of the others Subsequent analysis was then carried out by pooling the data from the two ranges of motion studied These pooled data are plotted in Fig 2.

Biceps brachii had a significantly shorter EMD (28 vs 38 ms) in the ECC condition at the faster than at the slower speed ($P<0\ 0?$) The velocity effect for triceps brachii was not significant in the pooled data although this muscle did show a significantly ($P<0\ 05$) shorter EMD in the fast than in the slower CONC condition at the FE 135-? joint range of motion (see Table 1) the range at which this muscle was stretched more during the ECC condition Neither muscle showed an effect in EMD attributable to velocity of movement in CONC

Although there was a statistically significant effect of type of contraction (CONC or ECC) on EMD this effect depended upon the velocity and on the muscle observed (see Fig. 2). Biceps brachii showed a significantly shorter EMD (28 vs. 41 ms) in ECC than in CONC of the faster movement ($P<0\ 001$) In fact the EMD of the fast ECC reached the same time as that of triceps muscle. There was no difference between CONC and ECC EMDs for biceps at the slower velocity nor for triceps at either of the two velocities of movement.

There was a statistically significant difference in EMD between biceps and triceps with no interaction with velocity or range of movement. This difference did depend however on whether the contraction was CONC or ECC

The appropriately combined data are in Table 2. Biceps had a significantly longer EMD than triceps (41 vs 26 ms) in CONC ($P<0\ 001$) but the EMDs were the same in ECC condition (33 vs 30 ms)

In an effort to explain these rather complex interactions of the effects on EMD of muscle, contraction type and velocity the duration of coactivation of EMGs (if any) of the accelerating and decelerating muscle was studied These times are listed in Table 2 and show that when biceps acted eccentrically to decelerate the movements its EMG activity appeared on average some 18 ms before the EMG activity from the accelerating muscle (triceps) ceased When triceps acted eccentrically and biceps concentrically no such EMG coactivation was observed

DISCUSSION

Normally the movement in joints is started by the isometric type of contraction so that the contractile component (CC) is first shortened stretching the series elastic component (SEC) The actual external movement occurs when the force with which CC pulls SEC exceeds that of the load (e g Bram... et al 1967) In the present expt CONC condition was designed to correspond to this kind of movement Another typical behavior of muscle is its...

2. Electromechanical delay and duration of coactiv tion (Coact+) or separation (Coact−) between f accelerating EMG and beginning of decelerating EMG averaged over range of motion and peak elocity of forearm

Concentric		Eccentric			
		Biceps		Triceps	
Biceps EMD ms	Triceps EMD ms	EMD ms	Coact ms	EMD ms	Coact, ms
41	26	33	+18	30	−7 0
13.1	10.5	13.2	31.1	6.9	13 9
39	40	40	40	36	36

stretch-shortening cycle in which a muscle is stretched by an external force such as gravity nertia of a moving limb or by its own antagonist cle. In the present study ECC condition was ght to represent this kind of movement.

e following findings are of importance from the ent study (1) In ECC, EMD of biceps was ter in fast as compared to slow contraction d, (2) In CONC condition EMD was longer for biceps brachii than for the triceps brachii Ex ations for the observed findings can be sought the possible structural differences between the muscles and from the behavior of the series tic component (SEC) in the contractions under y

general first, the results for EMD are in close ement with the lag time of 30–40 ms reported by ton et al. (1976) for the quadriceps femoris p. However in passive oscillation movement e forearm Komi & Cavanagh (1977) reported Ds of the order of 50 ms for the elbow flexor cles. That value is considerably longer than Ds in either CONC or ECC condition of the ent study These discrepancies in EMD values be explained through differences in the shold levels to which the force had to increase re the onset of tension development was re led. In the present study the beginning of ten- build up was taken from the onset of limb leration and not from a force threshold level of N (recorded at the wrist) as used by Komi & anagh (1977).

s suggested by Komi & Cavanagh (1977) the of shortening of the muscle s series elastic com- ent (SEC) might be a primary cause for the e of EMD in a given muscle Our results sup- this hypothesis primarily for the biceps brachii muscle in which case the EMD was shorter in fast ECC than in any other condition of that muscle. In faster ECC SEC is stretched at a faster rate which is likely to cause the force/length curve of that component to reach the required threshold level sooner The absence of the expected velocity effect on EMD in CONC is probably due to the small differences in limb velocities at the time when EMD is over in the two conditions of CONC contraction. In CONC the measured velocities represent the peak forearm angular velocities and not the muscle linear velocities in the very early stages of contrac tion whereas in ECC the peak angular velocities are directly related to the linear velocity of the muscles (Pertuzon & Bouisset 1971)

EMD includes both the events leading to the acti- vation of the cross-bridges and the time spent by CC for stretching SEC Although the latter gives a greater contribution to the total EMD the influence of the activation processes cannot be excluded This becomes important when one compare dif ferent types of muscle with respect to their rates of Ca^{2+} release from the sarcoplasmic reticulum to in- itiate the cross-bridge formation. A fast type muscle has been shown to release Ca^{2+} at a faster rate than the slow muscle (Harigaya et al. 1969) Upon relaxa- tion the uptake of Ca^{2+} by the sarcoplasmic retic ulum appears to take place faster in FT-muscle and its rate is related to the rise time of the isometric twitch contraction (Brody 1976). The rate of cross- bridge cycling is much lower in slow muscle fiber resulting in a lowered maximum rate of rise of iso- metric tension (Lännergren 1976) On motor unit level it has also been demonstrated that fast and slow units have different mechanical characteristics so that the force-time curve of the FT unit has both shorter rise time and shorter ½ relaxation time than

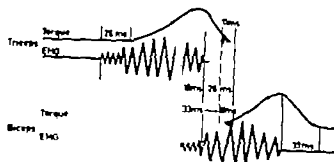


Fig 3 Schematic diagram of EMGs from movement accelerated from rest by triceps and decelerated by biceps brachii and accompanying hypothetical muscle torque (or force) time curves Times are from averages of observations summarized in Table Sketch shows 18 ms of biceps/triceps EMG coactivation and at least 11 ms of competing torque outputs of antagonistic muscles

that of the ST unit (Gydikov et al 1976) Consequently *Viitasalo & Komi (1978)* were able to show that a muscle containing predominantly fast type fibers (or motor units) is able to reach a certain submaximal force level sooner than the same muscle with higher percentage of slow twitch fibers

In the present study it was also hypothesized (see *Introduction*) that a muscle comprised of a preponderance of fast twitch fibers should yield shorter EMDs No analysis of the fiber composition of the subjects biceps and triceps brachii were performed in this study However studies on the force rise times (Buchthal & Schmalbruch 1970) on force–velocity relations (Jorgensen 1974) and on histochemical typing of the individual fibers (Johnson et al 1973) indicate that the triceps brachii is "faster" type muscle than the biceps brachii muscle Thus it seems likely that the different EMDs observed for the two muscles can be explained by the differences in their fiber structure Investigations are in progress to test this hypothesis more thoroughly with one muscle group and with wide range of fiber compositions

In fast ECC the biceps had essentially the same EMD as triceps Moreover biceps EMG activity in this condition usually commenced before triceps EMG had ceased There was rarely biceps/triceps EMG coactivation when triceps contracted eccentrically (Table 2) These observations point to the possibility that muscle spindle activity may influence the rate of passive stretching of SEC thereby affecting the duration of EMD From animal studies it is known that even in predominantly fast muscles the spindles are restricted primarily to those parts of the muscle which contain mainly slow fibers (Meyer Lohmann et al 1974) Further more muscles classified as slow types have higher spindle indexes than the fast ones (Diamond & Abrahams 1975) If the biceps and triceps muscles of humans also contain different distributions of muscle spindles this differentiation [illegible] have some functional role which causes the [illegible] muscles to have similar EMDs in fast ECC contractions Thus it is possible that spindle activation [illegible] more radily augmented in biceps brachii during fast stretches It must be pointed out however that the time sequences during ECC contraction [illegible] short which suggests that also Gamma-activation [illegible] may be augmented more in biceps than triceps

An alternative explanation of the shortened EMD in biceps brachii during fast ECC contraction [illegible] that in fast stretching the slow type muscle [illegible] capable of efficient storage of elastic energy and its utilization during the subsequent contraction phase of the stretch shortening cycle Such a phenomenon and the possible role of muscle fiber composition has been discussed by Komi & Bosco (1978) [illegible] respect to a normal jumping movement. It is conceivable that muscle spindle activity is related [illegible] this elasticity storage and utilization or indeed [illegible] there are even structural differences between [illegible] and slow muscle which account for the observa[illegible] tions Until more basic information is obtained about structural differences in fusimotor and different innervation between fast and slow muscle [illegible] their functional role in the final motor act [illegible] difficult to comprehend

The observation of EMG coactivation of biceps and triceps muscles when biceps contracted [illegible] centrically (Table 2) implies at first glance, [illegible] efficient cocontraction of antagonistic muscles Corser (1974) suggests however that the electro[illegible] mechanical delays act in such a way that [illegible] though electrical coactivation may be observed [illegible] there is no competing joint torque output [illegible] antagonist muscles His data show contraction [illegible] relaxation delays of approximately the same [illegible] duration This seems reasonable if one considers that at fast contraction speeds the muscles are [illegible] low force levels on the force–velocity curve The [illegible] in relaxation the time for muscle force to fall to [illegible] from a relatively low peak will be shorter than for a high peak force atta ed in a slower contraction [illegible]

Fig 3 is a hy etch of the [illegible]

[illegible]onships between concentric and eccentric [illegible]s, EMG coactivation time and muscle torque [illegible]n based on average delay times listed in Table [illegible]e sketch is of a forearm extension movement [illegible]erated by triceps and decelerated by biceps [illegible] with the assumption that contraction and [illegible]ation EMDs are about the same length. The [illegible]s shows that although biceps EMG activity [illegible] 18 ms before triceps EMG had stopped (i.e. [illegible]s of coactivation) there were 11 ms when [illegible] outputs were competing antagonistically. In [illegible], the cocontraction time was probably longer [illegible] the 33 ms identified as biceps EMD time was [illegible]ted at the time biceps torque exceeded that [illegible]ceps. Our data show, contrary to Corser's [illegible]) suggestion, the possibility not only of [illegible]ponent EMG coactivation, but also of compet[illegible] muscle torque outputs. Electromechanical delay [illegible] do, however, complicate the interpretation of [illegible]e relationships among EMGs.

[illegible]mmary electromechanical delays in the or[illegible] of 25–45 ms were observed. Biceps delays were [illegible]ively long in concentric contractions but in [illegible]tric contractions approached the EMD times [illegible]iceps. The latter was short in both concentric [illegible]ccentric contractions. The EMD time of biceps [illegible] shorter in fast than in slow movements in the [illegible]tric condition giving some support to the [illegible]thesis that the rate of stretching of the series [illegible] component of the muscle is the primary de[illegible]nant of EMD. The time of the delay, however, [illegible]fected by complex interaction of muscle fiber [illegible] composition, whether the contraction is con[illegible]ric or eccentric and the velocity of contraction. [illegible]ddition it is possible that gamma system in[illegible]ement is related to EMD time differences be[illegible]en muscles. The observation of triceps/biceps [illegible]G coactivation, although not central to the [illegible]y, when viewed with respect to electro[illegible]hanical delays in muscle torque output, [illegible]s yet another complication in interpreting EMG [illegible] relationships. We agree with Corser (1974) [illegible]t interpretation of antagonist EMG coactivation [illegible] mutually inhibiting muscle force or torque out[illegible] can lead to erroneous conclusions about human [illegible]ing mechanisms if electromechanical delays are considered.

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daptive changes in cerebral blood flow and oxygen consump-n during ethanol intoxication in the rat

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HEMMINGSEN R. & BARRY D I Adaptive changes in cerebral blood flow and oxygen consumption during ethanol intoxication in the rat. Acta Physiol Scand 1979 106 249–255 Received 28 Nov 1978. ISSN 0001-6772. Department of Psychiatry Department of Neurology and Psychochemistry Institute Rigshospitalet, Copenhagen, Denmark.

Cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$) were measured during acute and long-term ethanol intoxication in the rat. The purpose was to investigate whether the adaptive changes (development of tolerance) occurring in the CNS during ethanol intoxication were associated with changes in CBF and/or $CMRO_2$ Consistent with other studies we found that acute severe ethanol intoxication (median blood alcohol concentration (BAC=5 4 mg/ml)) caused significant decrease in CBF and $CMRO_2$. After 3–4 days of severe intoxication (BAC of 6.6 mg/ml) these physiological variables were less affected indicating that functional tolerance had developed $CMRO_2$ and CBF during acute ethanol intoxication were 9.3 ml/100 g/min and 60 ml/100 g/min respectively after the long term intoxication period these variables reached 11.2 ml/100 g/min and 78 ml/100 g/min respectively values not significantly lower than those of the control group. After induction of hypercapnia ($PaCO_2$ about 80 mmHg) CBF increased by 360% in the control group in the acutely intoxicated group CBF increased by only 127% and in the long term intoxicated group by 203% indicating that the cerebrovascular CO_2-reactivity had also adapted to the ethanol intoxication. It is concluded that adaptive changes of the CNS to chronic ethanol intoxication comprise alterations in $CMRO_2$, CBF and cerebrovascular reactivity

Key words Ethanol intoxication, tolerance physical dependence cerebral blood flow cerebral oxygen consumption, cerebrovascular reactivity

uired drug tolerance means that an increased unt of drug is required to produce the same ified degree of effect or that less effect is produced by the same dose of drug (Kalant et al 1971) physical dependence means that as a consequence of exposure to a drug the presence of that is required for normal function (Goldstein et 974), i e. to avoid development of a withdrawal drome Acquired tolerance and development of sical dependence represent adaptive changes that the organism can function "normally" in presence of the drug. If the drug is withdrawn er than the adaptation can be reversed a withdrawal syndrome develops.

The ability of ethanol to cause a change in tolerance and to induce physical dependence has been monstrated experimentally both in man (Isbell et 1955 Mendelson & La Dou 1964) and in animals (Majchrowicz 1975 Majchrowicz & Hunt 1976 for review see Kalant et al. 1971 Goldstein 1976)

Previous studies of CNS-changes during and after chronic ethanol administration have mainly included animal behaviour (Kalant et al 1971 review) or biophysical and biochemical changes (Blum & Wallace 1974 Griffith et al. 1974 Hunt & Majchrowicz 1974 Pohorecky et al 1974 Athee et al 1975 Goldstein 1976 Kalant 1978) Only occasionally have physiological variables such as EEG or seizure threshold been used (Wikler et al 1956 Kalant et al. 1971), and hence there is a need for investigation of possible pathophysiological changes in the brain metabolism as a whole

The purpose of the present study was to investigate whether the adaptive changes occurring in the CNS during ethanol intoxication were associated with changes in the physiological variables cerebral oxygen consumption ($CMRO_2$) and/or cerebral blood flow (CBF) which reflect neuronal functional level of the brain (Siesjö 1978).

Table 1 *Mean arterial blood pressure (MABP) arterial blood gases arterial pH cerebral blood flow (CBF) cerebral oxygen consumption (CMRO₂) and cerebral vascular resistance (CVR) during normocapnia in control rats and rats during acute and long-term ethanol intoxication*

Medians and quartiles are given n = number of animals BAC = blood alcohol concentration

	MABP (mmHg)	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	pHa	CBF (ml/100 g min)
Control n = 6	150 (145–152)	37 (35–38)	127 (118–144)	7 39 (7 37–7 39)	95 (81–123)
Acute intoxication n = 6 BAC = 5 4 mg/ml	104 (82–120)	39 (36–43)	136 (121 153)	7 30 (7 23–7 37)	60 (57–77)
Chronic intoxication n = 7 BAC = 6 6 mg/ml	128 (117 135)	38 (35–39)	135 (107–137)	7 41 (7 38–7 46)	78 (65–89)

P<0 05 P<0 01

METHODS

The experiments were performed on Wistar rats 2 300 g which had prior free access to water and food pellets CBF and $CMRO_2$ were measured during normocapnia and hypercapnia in 3 groups of animals (1) no intoxication n = 6 (2) acute intoxication n = 6 (3) chronic intoxication n = 7

Acute intoxication was induced by a single gastric intubation with 20% W/V ethanol in Ringer solution containing 30% W/V sucrose each rat receiving 7–9 g ethanol/kg.

Chronic intoxication was induced by repeated gastric intubation (using the above ethanol solution) 4 times a day for 3–4 days the daily dose being 9–12 g/kg. In addition the rats received 0 5 ml multivitamin solution daily

The clinical state of intoxication was assessed according to the following system adapted from Majchrowicz & Hunt 1976

Neutrality No signs of intoxication

Sedation Reduced muscle tonus dulled appearance slow locomotor activity no impairment of gait or coordination

Ataxia 1 Slight gait impairment and slight motor incoordination still elevation of abdomen and pelvis

Ataxia 2 Clearly impaired staggering gait and motor coordination some elevation of abdomen and pelvis

Ataxia 3 Slowed righting reflex heavily impaired motor coordination no elevation of abdomen or pel i

Loss of righting reflex (LRR) Unable to right itself when placed on its back. Other reflexes present Little spontaneous motor activity

Coma (general anaesthesia) No signs of movement no response to pain stimuli no eyeblink reflex spontaneous breathing.

The intubation dose was set according to the clinical evaluation the object being to keep the animals in a tate of severe intoxication (ataxia 3—LRR) which is known to cause development of a physical dependence which produces a severe withdrawal syndrome on termination of ethanol administration (Majchrowicz 1975 Hemmingsen et al 1979c) Thus a neutral animal received 4–5 g/kg whereas an animal with LRR received 0–1 g/kg at ... intubation

Following the intubation (acute group) or the last intubation (chronic group) 1–2 h elapsed before ... preparation of the animals for CBF/$CMRO_2$ measurements at which time the blood alcohol concentration (BAC) was determined The animals were weighed before and after the intoxication period

The measurement of cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$) was performed using the intraarterial 133-Xe injection technique (Hertz et al ... Hemmingsen et al 1979a) The non-intoxicated rats ... operated under halothane anaesthesia, 0.8% in 70% ... 30% O_2. After completion of surgery the halothane ... switched off the animals immobilised with ... choline 20 mg/kg i v In the intoxicated groups ... was operated upon unless it was in a state of ... intoxication and all animals received 70% N_2O 30% ... as a supplementary anesthetic. The rats were ... cheotomized and artificially ventilated

Rectal temperature was kept close to 37°C (±0 ...) by means of a heating blanket A catheter was inserted ...

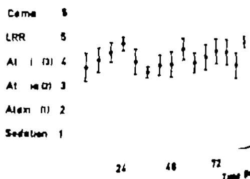


Fig 1 Clinical status during the ethanol intoxication period The clinical states sedation-coma are numbered 1–6 and the mean and standard deviations are given concerning the state of in... at each 6 h evaluation

…mm)	CVR (mmHg (ml/100 g/min)$^{-1}$)
)	1.52 (1.20–1.90)
)	1.75 (1.52–1.86)
)	1.34 (1.22–2.15)

…al artery for continuous measurement of mean ar… blood pressure (MABP) and for sampling of blood to …ure arterial pH, PaO_2 and $PaCO_2$. MABP was kept …±10% of the initial value by transfusion of fresh blood from ethanol intoxicated rats. To avoid ex…rebral contamination of 133-Xe clearance curves, all …cerebral branches from both carotid arteries were …d. A polyethylene catheter was inserted in retro… direction into the right external carotid artery, its tip placed at the carotid bifurcation to avoid obstruction …od flow into the internal carotid.

…rebral venous blood was sampled from … 3 cm cannu…rted into the torcular sinus through a burr hole in the … On completion of the surgical preparation which … about 40 min the animals were left undisturbed for … and $PaCO_2$ was adjusted to 38 mmHg by altering …spiratory volume. CBF and $CMRO_2$ were first meas… during normocapnia and then 15 min after inducing …capnia (by adding CO_2 to the inhaled gas mixture …ing $PaCO_2$ to 80 mmHg).

…measurements were made in duplicate and the val… averaged.

…F was calculated from the initial slope of … semi-…logarithmical plot of the externally detected 133-Xenon …ance curve after intracarotid injection of … 10 µl of 133-Xenon dissolved in saline (3.5 mCi/ml). For …culation of $CMRO_2$, arterial and cerebral venous …d was sampled concomitantly within 30 … after 133-…n injection and the oxygen content of these samples …ured (Borgström et al. 1974). To obtain the $CMRO_2$ …arteriovenous difference in oxygen content was mul…ied by the CBF value. Cerebral vascular resistance …R) was calculated as the ratio MABP/CBF which is an …estimation as the cerebral venous pressure (unknown) …ld be subtracted in the numerator. Blood alcohol con…trations (BAC) were determined by the enzymatic …od measuring the increase in NADH produced in the …hol dehydrogenase reaction.

…tistical differences were tested by non-parametric …ods using Wilcoxon … test for paired data within …ps and Mann-Whitney … test for differences between …ps.

RESULTS

The intoxication

The degree of clinical ethanol intoxication in the long-term intoxicated group is expressed in Fig. 1 which shows that the animals were severely intoxicated during the whole period.

The median weight loss in the long-term intoxicated group was 15% (quartiles 1 –17%).

At the time of CBF and $CMRO_2$ measurements the animals in the chronic intoxication group had a median BAC of 6.6 mg/ml (quartiles 6.5–6.9 mg/ml) whereas the acutely intoxicated animals representing the same degree of clinical intoxication had a median BAC of 5.4 mg/ml (quartiles 4.9–6.0 mg/ml). The difference in BAC between the two groups was statistically significant ($P<0.05$).

The measurements of cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$)

The measured and calculated physiological variables in the acutely intoxicated group, the long-term intoxicated group and the control group during normocapnia and hypercapnia are given in Tables 1 and 2 respectively; these tables also show the results of statistical comparison between the intoxicated group and the control group.

In each group the induction of hypercapnia ($PaCO_2$≈83 mmHg) was followed by a decrease in arterial pH of about 0.30 whereas MABP and PaO_2 were practically unchanged (the rats always had a PaO_2 above 100 mmHg). After induction of hypercapnia CBF increased significantly in all 3 groups ($P<0.01$). $CMRO_2$ did not change significantly in the control group or the long-term intoxicated group but in the acutely intoxicated group median $CMRO_2$ decreased by 44% ($0.05<P<0.10$).

Comparison between the experimental group

During normocapnia CBF in the acutely intoxicated group was 39% lower than in the control group whereas CBF in the long-term intoxicated group did not deviate significantly from the control CBF-value. During hypercapnia CBF in both the intoxicated groups was lower ($P<0.01$) than in the control group. Further the increase in CBF after induction of hypercapnia was significantly smaller in the intoxicated groups than in controls. In the control group CBF increased by 360%, in the acutely intoxicated group by 177% ($P<0.02$) and in the long-term intoxicated group by 203% ($P<0.05$). MABP

Table 2 *Mean arterial blood pressure (MABP) arterial blood gases arterial pH cerebral blood flow (C... cerebral oxygen consumption (CMRO$_2$) and cerebral vascular resistance (CVR) during hypercapnia in co... rats and rats during acute and long term ethanol intoxication*

Medians and quartiles are given *n* = number of animals BAC = blood alcohol concentration

	MABP (mmHg)	PaCO$_2$ (mmHg)	PaO$_2$ (mmHg)	pHa	CBF ([illegible])
Control *n* = 6	154 (149–158)	81 (78–87)	133 (130–135)	7 10 (7 08–7 11)	[illegible]
Acute intoxication *n* = 6 BAC = 5 4 mg/ml	100* (90–118)	87 (85–90)	129 (123–138)	7 01 (7 00–7 07)	[illegible]
Chronic intoxication *n* = 7 BAC = 6 6 mg/ml	133 (1_3–140)	82 (80–85)	144 (135–149)	7 18 (7 15–7 20)	[illegible]

P<0 05 *P*<0 01

was lower in the intoxicated groups both during normocapnia and hypercapnia During normocapnia CVR in the intoxicated groups did not deviate significantly from controls although there was a trend to a higher value (13%) in the acutely intoxicated group After induction of hypercapnia CVR was significantly higher in both the acutely intoxicated group (131%) and the long term intoxicated group (61%) as compared to the control group CMRO$_2$ was significantly lower in the acutely intoxicated group compared to controls both during normocapnia and hypercapnia whereas such a difference did not appear in the long term intoxicated group

PaCO$_2$ PaO$_2$ and arterial pH in the intoxicated groups did not differ significantly from values in the control group neither during normocapnia nor during hypercapnia (but there was a trend to a lower arterial pH in the acutely intoxicated group)

DISCUSSION

A comparison between the cerebrovascular effects of acute and long-term intoxication has not been reported previously Battey et al (1953) measured CBF and CMRO$_2$ in man using the Kety Schmidt (1948) method and found that a mild ethanol intoxication (mean BAC = 0 68 mg/ml) did not cause major changes in these variables this was confirmed by Fazekas et al (1955) studying patients with a mean BAC of 1 4 mg/ml In patients admitted during severe ethanol intoxication (coma or stupor) with a mean BAC of 3 ? mg/ml Battey et al (1953) found a CMRO$_2$ which was 30% below the value encountered after recovery CBF was about 30% higher than after recovery Hadji-Dimo et al (1... demonstrated a biphasic effect of ethanol i... brain i e CBF transiently increased at low l... (0 5 mg/ml) and it decreased during further ... ministration of ethanol These CBF changes ... lelled changes in the electroencephalogram ... authors summarized that ethanol in low conce... tion caused increased functional activity ... while ethanol in higher concentration cause... well-known depression in functional activity ... pressor effect of ethanol upon cerebral oxygen ... sumption was also found in in vitro studie... Fuhrman & Field (1948) These authors also ... indications of a biphasic effect with incr... oxygen consumption at lower ethanol conce... tions Severe ethanol intoxication comm... causes a decrease in blood pressure (Battey ... 1953 Fazekas et al 1955 Hadji Dimo et al ...

The findings in the present investigation ... accordance with the results of most of the s... mentioned above Acute severe ethanol int... tion caused a reduction in CMRO$_2$ and MAB... cording with the finding of Hadji-Dimo et al ... who studied cats there was a decrease in CB... this finding is at variance with the results of ... et al (1953) (see above) The patients in their ... were breathing spontaneously and were ... capnic with an arterial PCO$_2$ of 47 mmHg co... to the 39 mmHg measured after recovery ... difference in arterial PCO$_2$ may well expl... 30% higher CBF during intoxication as it is ... that CBF increases by about 4% per mm ... crease in arterial PCO$_2$ (Olesen 1974)

The present results from the acute and lon... intoxication groups te a clear pattern

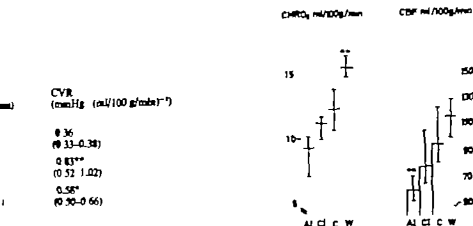


Fig 2 Cerebral oxygen consumption ($CMRO_2$) and cerebral blood flow (CBF) in rats during acute ethanol intoxication (*AI* =6), chronic intoxication (*CI* =7), controls (*C* =6) and during state of severe ethanol withdrawal (*W* =7) The findings show that $CMRO_2$ and CBF adapts towards normal during severe chronic ethanol intoxication and that they are elevated above normal during ethanol withdrawal thus reflecting the rebound hyperactivity of the CNS during that state ($PaCO_2$=35–38 mmHg.) Medians and quartiles are given. $P<0.05$ $^{**}P<0.01$ when compared with control. Values during ethanol withdrawal taken from Hemmingsen et al. 1979c

...rmocapnic CBF and $CMRO_2$ in the acutely ...cated group were significantly lower than in ...ntrol group whereas such a difference was not ...rable in the long-term intoxicated group· this ...he case in spite of the fact that BAC was ...cantly higher in the long-term intoxicated ... The tendency to more severe effect upon the ...ological variables during acute intoxication ... lower BAC was also seen during hypercapnia ... CBF $CMRO_2$ and CVR significantly de... from controls· during hypercapnia CBF and ...n the long-term intoxicated group also de... from control values but to a lesser extent ...n the acutely intoxicated group Similarly ...in the acutely intoxicated group deviated from control value both during normocapnia ...ypercapnia.

...n the CBF-response to increased arterial ...is considered we again found that this re...e was smaller in the acutely intoxicated group compared to the long-term group

...our opinion the differences between the two ...al intoxicated groups reflect the development ...*nctional tolerance* i.e changes in properties ...functions of the target tissue which render it ...nsitive to exposure to the drug (Kalant et al. ...) In an earlier study (Hemmingsen et al 1979c) ...und that $CMRO_2$ was increased during ethanol ...drawal (physical dependence) in the rat and the ...nt findings indicate that this variable also re...s the adaptive changes underlying development ...actional tolerance.

...n acute intoxication with a central depressant ...es a decrease of $CMRO_2$ which is commonly ...rpted as reflecting neuronal functioning level of the brain (Lassen 1959 Ingvar 1977) When the intoxication is prolonged the brain adapts to this by mechanisms which express themselves via a trend towards normalisation of $CMRO_2$. When the depressant is suddenly removed the adaptive changes persist for some time and this can be measured as an abnormally high $CMRO_2$ reflecting a rebound hyperactivity in brain function. The adaptive change and the rebound hyperactivity reflected through changed $CMRO_2$ and CBF values is illustrated i Fig. 2

We shall finally comment on two more points. Whereas the lower $CMRO_2$ during acute ethanol intoxication must be considered a direct reflection of changed cellular function the concomitant lower CBF was most probably an event secondary to the $CMRO_2$-decrease the existence of such a metabolic control of CBF in the rat has been shown to occur during various other conditions, e.g. immobilization stress (Carlsson et al 1977) or beta-adrenergic receptor blockade (Hemmingsen et al 1979a)

The lower MABP during ethanol intoxication could explain the lower CBF i.e If cerebral autoregulation (the ability to maintain an unchanged

CBF at a changed MABP) was disturbed Data on cerebral autoregulation during ethanol intoxication in the rat are not available but Hernández et al (1978) found in rats during nitrous oxide anaesthesia that autoregulation was effective from a MABP of 80 mmHg to 160 mmHg Hence it seems improbable that cerebral autoregulation should be lost at a MABP of about 100 mmHg which was the MABP level measured in the acutely intoxicated group in the present study

In the acutely intoxicated group there was a de crease in $CMRO_2$ during hypercapnia which was not seen in the other groups Berntman et al (1978) found that hypercapnia with an arterial PCO_2 of 70–80 mmHg caused an increased $CMRO_2$ in rats anesthetised with nitrous oxide and this was confirmed in a study by Hemmingsen et al (1979*a*) At higher values of $PaCO_2$ Berntman et al (1978) found a decrease in $CMRO_2$ and the finding of a reduced $CMRO_2$ in the present study may be explained by a synergistic effect of the $CMRO_2$-depressing action of ethanol and the $CMRO_2$-depressing part of CO_2 s action at PaCO above 80 mmHg a similar trend to a decreased CMRO during hypercapnia of about 80 mmHg administered in combination with a central depressant was seen in an earlier study of pen tobarbital (Hemmingsen et al 1979*b*) and this may reflect a general rule that hypercapnia augments the effect of central depressants upon $CMRO_2$ The results of the present study indicate that in the case of ethanol this effect may be diminished because of adaptation during long-term intoxication

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tric acid secretion and drinking in the Atlantic cod *dus morhua*) during acidic or hyperosmotic usion of the intestine

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HOLSTEIN B. Gastric acid secretion and drinking in the Atlantic cod (Gadus morhua) during acidic or hyperosmotic perfusion of the intestine. Acta Physiol Scand 1979 106. 257 265 Received 18 Dec 1978 ISSN 0001-6772 Department of Zoophysiology University of Göteborg, Sweden.

Cods were equipped with cannulae for drainage of the stomach and for separate perfusion of the stomach and intestine Acidity volume, and osmolality of the gastric outflow were measured During perfusion of the intestine with near-isosmotic saline (1 part sea-water 2 parts distilled water 33% SW[1]) and the stomach with pure (100%[1]) SW gastric acid output was high and volume output slightly above the infused volume The osmolality of the gastric perfusate decreased during passage of the stomach. It was concluded that no drinking occurred, and that the decreased osmolality was due to dilution by gastric secretions and osmotically lost water When substituting the isosmotic intestinal perfusion to dehydrating perfusion (100% SW) gastric acid secretion was depressed but volume output was unaffected. Also perfusion of the intestine with acidified 33% SW depressed gastric acid secretion and in addition increased volume and osmolality of the gastric outflow The results suggest that perfusion of the intestine depresses the drinking reflex and that this depression is surmounted by intestinal acidification. Possible mediators of the intestinal feed-back mechanism for the inhibition of gastric acid secretion are discussed

Key words Drinking, fish physiology gastric acid secretion, intestinal acidification

e regulation of gastric acid secretion, the lic, the gastric and the intestinal phases are alized (Grossman 1967 Wright & Hirschowitz Cooperman 1977) The intestinal phase com- s both stimulatory and inhibitory components Rasmussen 1978) and has been the subject of mammalian studies Studies of gastric acid tion in submammalian species, excepting the led gastric mucosa from the frog, are in- ent, and concerning the intestinal phase the seems completely ignored.

e continuous drinking of the sea-water teleost tributed to wash gastric secretion out of the ulated codfish stomach. This technique pre sly used in secretory studies in this laboratory Holstein 1975 1976 1977) suffered from the serious dvantage to dehydrate the fish. To divert gastric etions from the intestine the pylorus was li- d and this prevented intestinal resorption of er and thus rendered replacement of water lost to the hyperosmotic environment impossible To permit this compensation the intestine was cannulated and perfused Perfusion of the intestine at a rate of about 8 ml/h completely inhibited drinking perfusion at a lower rate decreased drinking (Holstein 1979) These effects however were independent of the degree of dilution of the sea-water used for the perfusion, both dehydrating solutions (100% SW 67% SW[1]) and solutions permitting the fish to maintain its water balance (50% SW 33% SW[1]) inhibited drinking. To be able to collect gastric secretions in the absence of drinking, also the stomach was cannulated and perfused. It was found that gastric acid secretion in 100% SW or 67% SW-intestinally perfused fishes was low or absent and that 50% SW and 33% SW-perfused animals exhibited

[1] 100% SW indicates pure sea-water 67% 50% and 33% SW indicates mixtures of 100% SW and distilled water in the proportions 2 1 1+1 and 1+2, respectively

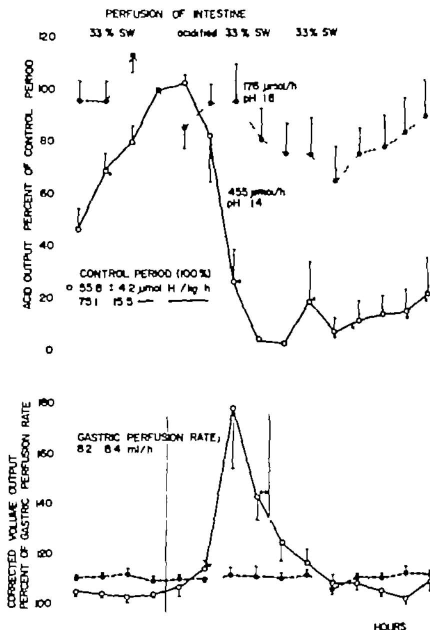


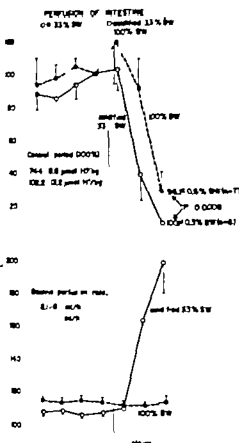
Fig. 1. Effect of intestinal acidification on gastric acid secretion (upper panel) and gastric volume output (lower panel). The group receiving the lower acid load comprised 8 animals, the group receiving the higher load 5 animals. Gastric perfusion with 100% SW-PR ... denotes a significant difference from the control h at the $P<0.05$, $P<0.01$ and $p<0.001$ level, respectively.

high rates of 'basal' acid secretion. As the intestine is continuously perfused, the technique appeared suited for studies of the intestinal phase of regulation.

The past decade has seen an enormous expansion in the knowledge of peptide hormones. Several peptides of intestinal origin have been isolated or proposed and their role as e.g. gastric secretory regulators is discussed (Grossman 1974, 1977, Rayford et al. 1976). Comparative studies comprising both structural and functional aspects should be ... to achieve a fuller understanding of the gut ... tides. This work, in indicating the existence of ... intestinal feedback mechanism for control of gastric acid secretion, provides some basis for further research on the function of gut hormones in fish.

MATERIALS AND METHODS

Codfishes of both sexes, weighing between 320 and ... were used. Before experiment, the fishes were ...



Effect of intestinal acidification (6 animals) and [illegible] perfusion of the intestine (7 animals) on acid secretion and gastric volume output. Gastric [illegible] with 100% SW–PR.

[illegible] aquaria (10°C) for at least one week. They were [illegible]. Surgery was carried out under MS-222 (Sandoz) [illegible] as described earlier (Holstein 1975). The [illegible] was equipped with two cannulae: one for drainage and one for infusion of perfusion fluid. Also the intestine was cannulated for perfusion. A detailed description of the surgical procedures has been given (Holstein 1979). [illegible] positioned were C1, C2, and C3.

[illegible] experimental schedule. Following recovery from anaesthesia the fishes were transferred to smaller tanks and [illegible] and intestinal cannulae connected to roller pumps. Perfusion started. The gastric drainage was divided in [illegible] fractions. The intestine was perfused with 33% SW, the stomach with 100% SW or 33% SW containing [illegible] mg/l phenol red (PR, sodium salt, Sigma). Perfusion rate for both stomach and intestine was about 8.5 ml/h [illegible] varied slightly between pumps and from one experiment to another. The actual rate was recorded following [illegible] experiment.

[illegible] fishes were left without further treatment for at [illegible] 20 h, and were then used for [illegible] maximum 3 expts. during 4 postsurgical days. Final body weight was then recorded and expressed as percentage of the presurgical b.wt. In different experiments, the intestinal perfusion was changed to 100% SW or to an acidic solution isosmotic with 33% SW ("acidified 33% SW").

Analyses and presentation of the data. In each fraction the concentration of PR was determined by reading the optical density (463 nm) of 200 μl aliquots diluted with 2.0 ml 0.1 M HCl. An identically diluted aliquot of the perfusion fluid served as standard. From the amount of PR entering the stomach, and the amount of PR found in the effluent, the recovery was calculated and the fraction volume accordingly corrected. 2-ml aliquots of the gastric effluence were titrated (Autotitrator assembly, Radiometer) with 0.05 M sodium hydroxide to the pH of the perfusion fluid. The acid content of each fraction was calculated from acidity and corrected volume. Acid output is expressed as μmol H/kg·h; in calculating this the b.wt. before surgery was used. In the figures, acid output has been normalized so that output for the h preceeding treatment ("control h") equalizes 100%. Gastric effluent rate is given as percentage of volume rate of the gastric perfusion. Osmolality was measured using Wescor 5100B Vapor pressure osmometer.

Preparation of acidified 33% SW. Hydrochloric acid, 0.17 M, in volume calculated to give the desired acidity in final volume of 2.0 l, was mixed with 33% SW to about 1.5 l. The osmolality was adjusted to that of 33% SW by addition of distilled water or 100% SW as required and made to volume with 33% SW. Final acidity was determined by titration to pH 7.0. Due to slightly different rates of intestinal perfusion the acid load to the fishes varied (means are given). The concentrations used were 51 (higher load) and 21.2 μmol H/ml (lower load).

Statistics. The nonparametric Randomization test for independent samples and the Kruskal-Wallis one-way analysis of variance (Siegel 1956) or the paired *t*-test were used for comparisons of means. To calculate the level of significance between two groups of means, the respective moments were pooled (Lehmann 1959). Differences were considered significant if $p<0.05$ was attained.

RESULTS

The effect of intestinal acidification on the gastric volume output is shown in Fig. 1. For the lower acid load no significant change was found. During the higher load, volume output increased in the second treatment h, attained a maximum in the 3rd h and then decreased in the 4th h.

Gastric acid secretion was only slightly affected by the lower acid load to the intestine (Fig. 1, upper panel); the change was significant only at the 3rd h following rechange of the intestinal perfusion to 33% SW. The higher load caused a profound depression of gastric acid output, lasting several h following rechange to 33% SW. Note that volume output returned to the basal level before acid output

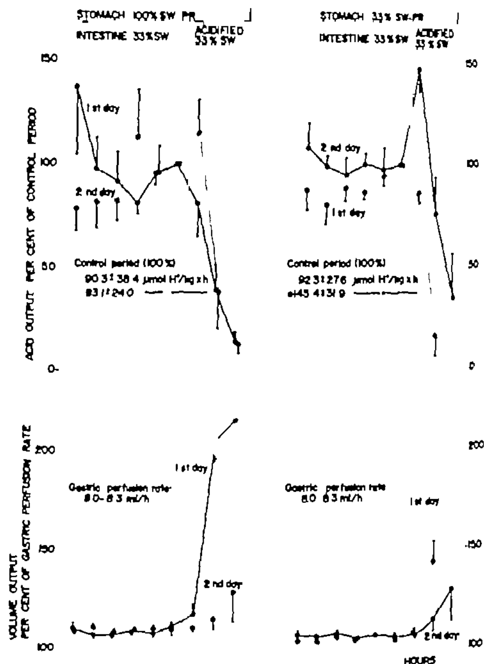
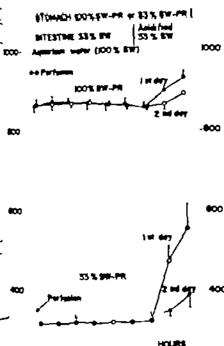


Fig 3 Gastric acid secretion and gastric volume output during intestinal acidification in two series of cods. In one series (●) the stomach was perfused with 100% SW–PR during the 1st day and with 33% SW PR during the 2nd day In the other series (O) the order of gastric perfusions was reversed For number of animals and statistical analyses refer to Table 1 and the text The osmolality of the gastric outflow is shown in Fig 4

tended to recover These fishes were killed 3 days later: their postexperimental b wt indicated that they were in proper water balance To obtain information as to a possible change in b wt during intestinal acidification the expt (higher load) was repeated and the animals killed following 3 h of intestinal acidification For comparison another series of fish was intestinally perfused with 100% SW a treatment known to dehydrate the fish (Holstein 1979)

Perfusion of the intestine for 3 h with 100% S[illegible] had no effect on the gastric volume output (Fig [illegible] lower panel) In the acid perfused group however volume output again increased (Randomization test $P = 0.0000$ the mean for all preacid h vs the mean for all treatment [illegible] to the acid secretion



/ Osmolality of gastric outflow before and during [in]testinal acidification and isosmotic (33% SW–PR) or [hyperos]motic (100% SW–PR) perfusion of the stomach [...] acid secretion and volume output are shown in [...]

[...]cation of the intestine was a more effective [...] than perfusion with 100% SW (Randomi[zation] test and pooling of the 3 treatment h [...]499)

[Th]e effect of elimination of the gastric tissue-to-[...] osmotic gradient was studied in experiments [descri]bed in Figs. 3–4 Two series of fish were used, [...] each the intestines were acidified for 3 h [...] load) on two occasions separated by 20 h [...] gastric perfusion fluid was 100% SW–PR on [one] occasion, and 33% SW PR during the other [...] perfusions were changed following the first challenge. One series received 100% SW–PR [duri]ng the first day and 33% SW during the second. [Th]e other series the order was reversed

[In] the basal state, i.e. in the period before intesti[nal a]cidification the volume of the gastric effluence [was] greater during gastric perfusion with 100% [SW]-PR than with 33% SW–PR (Fig. 3 lower [pan]els). The differences were statistically signifi[cant] irrespective of the subgroups compared (Ta-[ble] 1).

During intestinal acidification volume output again increased Inspection of Fig. 3 lower panels indicates that the volume increase was greater during the first acid challenge less during the second challenge during the following day and that the perfusion of the stomach whether hyperosmotic or isosmotic was of no or slight importance With two exceptions, the statistical analyses confirm this impression. Analysis of variance showed that a significant ($P<0.02$) difference existed among the four groups Further analysis employed the Randomization test and pooling to obtain the level of significance for differences during the whole acidification period Considering both gastric perfusions together volume output during the 1st day (1st day 100+33% SW PR) is significantly different ($P=0.008$) from the volume output during the second day (2nd day 100+33% SW–PR) Considering the groups separately a significant ($P=0.0330$) difference was found between 1st and 2nd days during gastric perfusion with 100% SW–PR, but not during perfusion with 33% SW–PR ($P=0.0739$) When instead, the values were grouped according to kind of gastric perfusion i.e. (100% SW–PR day 1+2) vs. (33% SW–PR, day 1+2) (100% SW–PR, day 1) vs. (33% SW PR, day 1) or (100% SW PR, day 2) vs. (33% SW–PR, day 2) no statistically significant differences were found In the first series of fish, the volume output during intestinal acidification was greatest during the first day ($P=0.0267$ for the comparison (100% SW–PR, day 1) vs (33% SW–PR day 2)) In the second series (33% SW PR, day 1 and 100% SW–PR day 2) no significant difference was found

As in the previous expts. the measured output of gastric acid decreased during intestinal acidifications (Fig. 3 upper panels) However considering the whole acidification period no significant difference among the 4 groups was found ($P>0.3$ analysis of variance)

In 4 fishes 2 during 1st day and 2 during 2nd day acidification, no increase in gastric volume output was detectable In the 3rd h of intestinal acidification gastric acid output did not exceed 7.2% of the control h in any of these 4 fishes.

Due to accidental dilution of the 100% SW–PR, the osmolality of this solution was slightly below that of the aquarium water (Fig. 4) During perfusion with 100% SW–PR, before intestinal acidification the mean osmolality of the gastric outflow was 867.3±8.1 mOsm/kg representing a decrease of

Table 1 *Gastric volume output in percent of the infused volume during 6 h preceding intestinal acidification (cf Fig 3)*

Values are mean ± S E for 6 h for the indicated number of animals[a]

Group volume output			Statistics[b]
1st day 33% SW–PR 104 68±0.36 (5)	vs.	1st day 100% SW–PR 108 96±0 79 (6)	$P=0\ 0000$
2nd day 33% SW–PR 106 62±0.54 (4)	vs.	2nd day 100% SW–PR 109 22±0 67 (4)	$P=0\ 0013$
1st day 33% SW–PR	vs	2nd day 100% SW–PR	$P=0\ 0000$
1st day 100% SW–PR	vs	2nd day 33% SW–PR	$P=0\ 0377$

[a] Each series initally comprised 6 animals Due to malfunction of gastric drainage the number was reduced during experiments

[b] Analysis of variance demonstrated a significant ($P<0\ 01$) difference among the 4 groups. The P-values were [illegible] by pooling the moments of hour-by-hour comparisons during the 6 h

about 70 mOsm/kg compared to the infused fluid During perfusion of the stomach with 33% SW–PR before intestinal acidification the osmolality of the outflow was close to that of the infused solution When perfusing the intestine with acidified 33% SW the osmolality of the gastric outflow increased the increases being greater during 1st day acidifications For the whole acidification period the differ ence (100% SW–PR day 1) vs (100% SW–PR day 2) was statistically significant ($P=0\ 0269$) The cor responding difference for 33% SW–PR perfusion was also significant ($P=0\ 0040$)

During intestinal acidification an increased viscosity of the gastric outflow was observed The change being most obvious during the 1st h of acidification was not quantified

DISCUSSION

Gastric volume output

Isosmotic intestinal perfusion Codfish equipped with a cannula to drain the stomach but gastrically and intestinally unperfused maintain a constant oral intake of water When starting the intestinal perfusion at a rate of about 8 5 ml/h drinking stops within 2 h (Holstein 1979) Although unlikely it could not be excluded that the effect on drinking was due to mechanical obstruction of the gastric drainage In the present work both the intestine and the stomach were continuously perfused In the basal state i e during perfusion of the intestine with 33% SW the gastric outflow volume was slightly greater than the infused volume indicating that fluid was added to the perfusate. This added volume was greater during perfusion of the stomach with the hyperosmotic solution than during iso[illegible] motic perfusion showing that the tissue-to-lumen osmotic gradient is responsible for at least part of the volume increase During isosmotic perfusion fluid was still added to the perfusate indicating that also gastric secretions contributes to the volume expansion The alternative explanation, that the fish maintains a slow oral water intake seems ex cluded by the decreased osmolality of the gastric outflow both during hyperosmotic and isosmotic gastric perfusion In the latter case, the difference in osmolality between aquarium water and perfu sion fluid is expanded and drinking if occurring should be easily disclosed Desalting of the sea wa ter by absorption of ions have been demonstrated in the Eel esophagus (Hirano & Mayer-Gostan 197[illegible]) but the present observation of decreased osmolality and a concomitant increase in volume rather sug gests that a hyposmotic fluid was added to the perfusate This fluid probably is a mixture of gastric secretions and osmotically lost water [illegible] tion of sea water in the teleostean intestine has been described by Smith (1930) and Sharratt et al. (19[illegible])

Hyperosmotic intestinal perfusion Change of the intestinal perfusion from 33% SW to pure sea-water dehydrated the fish but did not induce drinking This observation extends a previous one [illegible] commencement of hyperosmotic intestinal perfu sion in a drinking fish (intestinally unperfused) in hibits drinking, and is consistent with the sugges tion that the drinkin[illegible] [illegible]ed by [illegible]

olume- or pressure-sensitive receptors acti- when perfusing the intestine The estimation e drinking rate during gastric perfusion also „ the hypothesis that distension of the in- e should mechanically obstruct the gastric

ide intestinal perfusion When perfusing the tine with the higher acid load the gastric vol- output increased The response culminated in rd h and then declined despite continuing fication (Fig. 2) This makes it unlikely that the t as due to physical damage of the fish lead- o breakdown of the gastric mucosal barrier If ical damage had rendered the mocosa more then the high volume output should have sted. Furthermore loss of endogenous water e amount required should appreciably lower body weight This was not the case (Fig.) obviation of the tissue-to-lumen osmotic gra- should prevent or decrease the effect How provided comparisons are made between ay expts. or between 2nd day expts. no icant differences in volume output be hyperosmotically and isosmotically per groups during intestinal acidification were d (Fig. 3).

ice acidification of the intestine increased both me and osmolality of the gastric outflow it is ested that acidification induced oral intake water The alternative explanation, that the ach secretes a voluminous, highly hyperosmot uid seems unlikely although the increased vis- ty indicates that gastric secretions may contrib- to the effects. The conclusion is supported by ries of expts. (=6) in which phenol red was ded in the aquarium water but excluded from gastric perfusion. The experiments were carried in 2nd day acidifications During 33 % SW usion of the intestine, no PR was found in the uence but when the intestine was acidified marker appeared in the gastric outflow The erimental design allowed no correction for orption but the calculated amount of water ested compares very well with the slight increase ed in the reported 2nd day acidifications

The escape of the drinking from the acidification ulation (Fig 2) may be related to the lesser drink response encountered during the 2nd acid chal- ge (Fig 3). The mechanism which elicits drink t seems to incorporate an exhaustible step or are mpensating mech ted. Pancreatic secretion of bicarbonate might be a compensating reaction which removes or decreases the stimulus by neutralizing the intestine This requires, however that pancreatic secretion remains elevated for at least 20 h following intestinal acidification Perfusion of the intestine with acidified 33 % SW may stimulate drinking, or may inhibit the perfusion-induced depression of the drinking reflex. The mechanism remains to be elucidated

Gastric acid secretion

Confirming earlier findings (Holstein 1979) gastric acid secretion in intestinally 33 % SW-perfused cods was high This basal secretion was depressed by perfusing the intestine with a dehydrating (pure sea-water) solution Whether the secretory inhibition depends on the dehydration via a change in body fluid volume or composition or on the hyperosmotic challenge per se via release of an enterogastrone remains undecided Dehydration of frog and pigeon is known to depress acid secretion (Friedman 1939) Dehydration of the cod by simply leaving the intestine unperfused results in a non-secreting gastric mucosa (Holstein 1975 1976 1977 1979) but it cannot be excluded that the hyperosmotic stimulus in addition activates other mechanisms In man and dog, instillation in the duodenum of hyperosmotic saline inhibits gastric acid secretion (Sircus 1958 Ward et al 1969 Ward 1976) Vasoactive intestinal polypeptide (VIP) inhibits mammalian stimulated acid secretion (Makhlouf & Said 1975 Villar et al. 1976) and Ebeid (1977) demonstrated increased plasma levels of VIP following intestinal instillation of hyperosmotic saline in the dog. Bayliss & Starling (1903) reported the presence of a secretin-like factor in the Salmon intestine and a similar factor extracted from pike or cod intestine was found to more closely mimick the effects of VIP when tested on mammalian and avian pancreatic secretion (Dockray 1975 1976) Dockray (1976) also found that the peptide immunologically was closely related to VIP In a current work i.m injection of VIP has been found to inhibit gastric acid secretion in 33 % SW intestinally perfused cod. Studies on the role of this peptide in the gastric secretory inhibition elicited by sea-water in the intestine are in progress.

Acidification of the intestine with the higher load (about 450 μmol H /h) invariably depressed gastric acid output. The b wt. of the fishes killed during acid treatment (Fig 2) shows that no dehydration,

relative to presurgical status occurred However the b wt of 33% SW perfused fishes usually is 102–104% (Holstein 1979) Thus it is possible that intestinal acid perfusion caused a small water deficit either by impeding water resorption or by increasing secretions from the pancreas-intestine The b wt encountered 101 1±0 3% is close to that found in 50% SW intestinally perfused fishes (101 8±0 8%) and these later fishes exhibited a high rate of acid secretion (Holstein 1979) Thus neither the decreased acid output nor the increased volume output can be explained by dehydration but must be related to intestinal acidification by some other mechanism

The ingested sea water neutralizes part of the secreted acid and this complicates the evaluation of the results The neutralization cannot be neglected addition of an equal volume of 100% SW to an aliquot of gastric effluence resulted in a loss of 1 8 μmol H /ml Thus for a volume output of 200% about 29 μmol H would be lost (assuming a perfusion rate of 8 ml/h) However for three reasons the conclusion that intestinal acidification inhibits gastric acid secretion seems permitted (1) In 4 animals no drinking was elicited by the intestinal acidification still the decrease in acid output was the same (2) gastric output remains low despite return of the volume output to the basal level (Fig 1) and (3) gastric acid depression is similar during 1st and 2nd day acidifications despite great differences in drinking rate (Fig 3) To my knowledge this is the first demonstration of this feed-back mechanism in a non-mammalian vertebrate The finding that depression of acid output is the same with or without accompanying drinking tempts one to speculate whether the cod has exploited ingestion of sea water as an additional means to lower the gastric acidity

In mammals acidification of the duodenum inhibits (Sircus 1958 Konturek & Grossman 1965) while acidification of the jejunum stimulates (Ivy & McIlvain 1923 Sircus 1953 Konturek & Grossman 1965) gastric acid secretion The tendency for an initial stimulation of acid secretion during 2nd day acidifications could indicate a similar differentiation in the codfish intestine

Whether the inhibitory mechanism is nervous or hormonal remains to be elucidated In mammals intestinal acidification sets free secretin but the amount released seems to be too small to account for the acid secretory depression (Ward & Bloom 1974) Acid in the duodenum also releases CCK (Nakajima & Magee 1970 Reeder et al 1972) The hormone in some species, is a partial agonist for gastric acid secretion and therefore can act as an inhibitor (see Barrington & Dockray 1976). CCK-like activity has been extracted from the Eel intestine (Barrington & Dockray 1972) and Larsson & Rehfeld (1977) in the cod intestine found immunoreactivity indicating the presence of a molecule resembling both gastrin and CCK. The octapeptide of CCK depresses gastric acid secretion in 33% SW-intestinally perfused cod (Holstein to be published) The observed increase in volume of the gastric effluence during intestinal acidification if related to mucus secretion further indicates the participation of CCK and secretin (Vagne & Perret 1976 Kowalewski et al 1978)

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ase of gastrin from the skeletal muscles from the antral mucosa in cats induced ulfonuric drugs

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UVNÄS-WALLENSTEN K. EFENDIČ S UVNÄS B & LUNDBERG J M. Release of gastrin from the skeletal muscles and from the antral mucosa in cat induced by sulfonuric drugs. Acta Physiol Scand 1979 106:267 270. Received 18 Dec 1978. ISSN 0001-6772. Departments of Pharmacology Histology and Endocrinology Karolinska Institutet, Stockholm, and Karolinska Hospital, Stockholm, Sweden.

The present observations indicate that sulfonuric drugs release gastrin both from peripheral nerves in striated muscles and from endocrine-like cells in the gastrointestinal tract. Gastrin appears in perfusates of extirpated cat legs after administration of tolbutamide or glibenclamide (5–50 mg/kg or 5–900 μg/kg perfused tissue respectively) to the perfusion medium. Furthermore gastrin is released into the portal vein of cats after i adminstration of glibenclamide (5–50 μg/kg). The finding that sulfonuric drugs not only release insulin from β-cells in the pancreas, but also gastrin from gastrin producing cells in the stomach as well as from nerve fibers in the skeletal muscles indicate that sulfonuric drugs have more wide spread effects than previously assumed. Possible consequences of the drug induced release of peptides from peripheral nerves as well as of the release of gastrin from the gastrointestinal tract are discussed

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order to characterize the release mechanism of gastrinergic nerves putative gastrin releasing s were added to the perfusate Sulfonuric s, known to release insulin from the pancreatic cells, ere found to release gastrin from the cat preparation and as demonstrated in a second of the study also from the gastrointestinal tract.

METHODS

1 *Gastrin release from perfused legs*

Five cats were killed by Nembutal. The legs were extirpated above the knee or the elbow The popliteal or brachial arteries were cannulated and the legs were *hanged upsidedown* They were then perfused with Tyrodes solution at rate of 1 ml/min. Because of the position of the legs, the venous effluent dripped directly into the collecting tubes After 2–3 control periods (10 min each) tolbutamide (5 and 50 mg/kg perfused tissue) or glibenclamide (5–900 μg/kg perfused tissue) was added to the perfusion medium as bolus injection Immediately after administration of the drug, 5 samples were collected every 2nd min and then 4–5 samples were collected at 5 or 10 min intervals The samples were kept in an icebath before being boiled for 10 min They were then frozen until analyzed for gastrin

2 *Gastrin release from the antrum*

Six cats (2–4 kg) were anesthetized with chloralose and urethane (50 and 100 mg/kg). The trachea was cannulated, and the animals were allowed to breathe spontaneously Blood pressure was continuously monitored in a femoral artery The intestines below the duodenum, and the spleen were resected The superior mesenteric vein was

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2. Gastrin release from the antrum

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Table 1*a* *Release of gastrin (pg) into the perfusate of a cat leg in response to Tolbutamide 5 and 50 mg/kg*

Expt no	Tolbutamide (mg/kg)	Gastrin release (pg)
1	5	170
2	50	280
3	5	80
4	50	210
5	5	100
6	50	200
7	5	50
8	50	1 000
9	5	–
10	50	150
11	5	70
12	50	300

Table 1*b* *Release of gastrin (pg) into the perfusate of a cat leg in response to Glibenclamide 5, 50 and 500 µg/kg*

Expt no	Gliben-clamide (µg/kg)	Gastrin release (pg)
1	5	20
2	50	120
3	5	0
4	50	60
5	50	60
6	500	200
7	50	
8	500	150

cannulated and the portal vein was tied close to the liver Thus blood from the stomach the duodenum and the pancreas flowed backwards through the superior mesenteric vein. On its way to the cat s femoral vein it passed a dropchamber unit for quantitative recording of the blood flow The cats were heparinized to avoid clotting in the catheters.

Blood samples (5 ml) were collected proximal to the dropchamber unit Samples were taken at 20 and 10 min before and at 0 2, 5 7 10 20 30 and 60 min after injection of glibenclamide and the tubes were immediately put on ice After centrifugation plasma was taken off and frozen

To allow studies of *the intraantral* secretion of gastrin an acute antral pouch was created in two of the cats by tying ligatures round the pylorus and the antral-corpus border The antrum was then perfused with warm 0 15 M NaCl (pH-6) at a rate of 1 ml/min The perfusate entered the antrum via a catheter in the pylorus and left it via a small cannula inserted into the antral wall For further information of the surgical procedures see previous publications (Uvnäs et al 1975 Uvnäs-Wallensten et al 1976 Uvnäs-Wallensten 1977)

The antral perfusates were collected during 10 min periods pH was adjusted to 7 and the samples were boiled for 10 min before being frozen

The samples (blood and perfusate) were analyzed for gastrin according to Nilsson (1975)

To obtain the quantitative data of the gastrin release occurring into the circulation hormone concentrations were multiplied by the rate of plasma flow Analogously the output of hormones into the cat leg perfusate or into the antral perfusates was calculated by multiplying the concentration of hormones with the flow rate of the perfusion medium (1 ml/min)

RESULTS

Gastrin release from perfused legs

Tolbutamide 5 and 50 mg/kg perfused cat leg tissue given to the perfusion medium released gastrin into the perfusate The amounts released were 0–[illegible] and 150–1 000 pg respectively (Table 1*a*). The [illegible] release responses tended to be dose depende[illegible] Glibenclamide also released gastrin into the per[illegible] sate A small output was observed after administra[illegible] tion of 5 µg per kg perfused tissue (20 pg), whe[illegible] 50 and 500 µg/kg released 0–170 and 150–20[illegible] respectively (Table 1*b*)

The gastrin release occurred within 2–4 min [illegible] application of the drugs and subsided within [illegible] In some expts a second peak of gastrin wa[illegible] served towards the end of the expts. indica[illegible] biphasic release pattern (Fig 1) Only the gas[illegible] output contained within the first peak has been [illegible] cluded in the quantitative determinations.

Gastrin release from the antrum

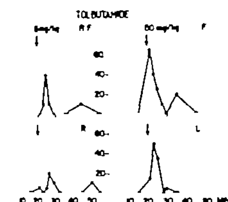
Five and 50 µg/kg of glibenclamide was give[illegible] bolus injections to 3 cats and in 3 additional c[illegible] single dose of 50 µg/kg was given

Both doses invariably caused a release of ga[illegible] into the circulation the amounts varying betw[illegible] 0 25–0 5 ng and 0 5–8 15 ng for the lower and [illegible] higher dose respectively (Fig 2 Table 2). In ex[illegible] in which two doses were given the higher d[illegible] tended to release 10–20 times as much gastrin a[illegible] the lower one

In 3 expts gastrin release into the lumen was [illegible] recorded Glibenclamide caused a concomitant [illegible] a parallel secretion of gastrin into the antral lu[illegible] (Fig 2 Table 2)

DISCUSSION

In the present study we have shown that gastr[illegible] released by sulfonyl[illegible] in cats Thus g[illegible]



Gastrin release (pg/min) into perfusate of the cat
r administration of Tolbutamide 5 and 50 mg/kg to
t fore and hindlegs and the left fore and hindlegs
ively

s in perfusates from extirpated cat legs after
istration of Tolbutamide and glibenclamide
mg/kg and 5–500 μg/kg perfused tissue
tively) and in the gastric venous outflow
l antral perfusates after i.v. injections of
clamide (5–50 μg/kg). It should be stressed
e amounts of sulfonuric drugs, used in the
t expts. are within the doses range used in
ent of diabetes.

gastrin which appears in the perfusate from
t legs after administration of Tolbutamide or
nclamide most probably comes from gas-
ic fibres in the sciatic and brachial nerves.

As mentioned in the introduction gastrin-17 is released into the perfusate upon electrical stimulation of these nerves (Uvnäs-Wallensten & Uvnäs 1978). In the cat mainly gastrin-14 circulates, whereas gastrin-17 is the major gastrin component in the antral mucosa and in the vagal nerves (Uvnäs-Wallensten et al. 1977).

These facts indicate that the cat leg gastrin is synthetized in nerves and is released from such gastrinergic fibres in the cat legs on stimulation of the nerves or following administration of sulfonuric drugs.

The function of the gastrinergic peripheral nerve fibres is as yet completely unknown. They may exert trophic effects on the muscles or they may be involved in the muscular contraction process. Thus it is well known that gastrin exerts myotonic effects on smooth muscles (Vizi et al. 1973) of the gastrointestinal tract.

It has been shown that sulfonuric drugs under certain conditions exert positive inotropic effects on the heart (for references see Levey et al. 1974). Since we have been able to release gastrin from a Langendorff heart preparation by adding glibenclamide to the perfusion medium (Uvnäs-Wallensten et al. to be published) the positive inotropic effects induced by these drugs may be caused by a release of gastrin or perhaps some other peptide from neurogenic stores in the heart.

2. *Release of gastrin (ng) into the gastric s outflow and into the antral lum n in re to glibenclamide 5 nd 50 mg*

xperiments. ithin the brackets have been performed same cats

Gliben-clamide (μg/kg)	Gastrin release (ng): Into gastric venous blood	Into the antral lumen
5 }	0.4	
50 }	8.15	
5 }	0.8	20
50 }	4.5	54
50	0.5	3
50	8.5	5
5 }	0.25	
50 }	1.9	
50	4.2	

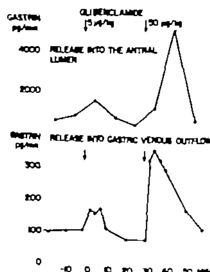


Fig. 2. Gastrin release (pg/min) into the antral lumen (upper panel) and into the gastric venous outflow (lower panel) after i. administration of 5 and 50 μg/kg of Glibenclamide.

The sulfonuric drugs also stimulate gastrin release from the GI tract This gastrin most probably to a major extent originates from the G-cells of the antro-duodenal mucosa However since gastrin has recently been demonstrated to occur in the vagal nerves it cannot be excluded that part of the gastrin comes from vagal nerve terminals (Uvnäs Wallensten et al 1977)

Nor can it be excluded that part of the gastrin releasing effect exerted by the sulfonuric drugs is caused by activation of the nerves that innervate the G-cells because the vagal innervation of these cells is presumably peptidergic (Uvnäs Wallensten & Andersson 1976)

It is a well known fact that treatment with sulfonuric drugs may cause gastrointestinal side effects Some of these side effects can perhaps be attributed to an increased release of gastrin and consequent effects on gastric acid secretion and gastric motility The amounts of gastrin released by Glibenclamide are in fact of the same order of magnitude as are the amounts of gastrin released by electrical vagal stimulation (Uvnäs Wallensten et al 1975)

An oral glucose load produces a higher insulin output than does glucose given i v The difference is thought to be due to the fact that the orally administered glucose also releases gastrointestinal hormones (such as gastrin) which in turn promotes the release of insulin Consequently the gastrin release induced by the sulfonuric drugs may be advantageous since it may contribute to the stimulation of insulin release induced by these drugs

It has been known since long that sulfonuric drugs cause a release of insulin from the pancreatic β-cells (Loubatières 1942) The present results show that sulfonuric drugs have a much wider spectrum of action than currently assumed The ability of the sulfonuric drugs to release several peptides from both endocrine like cells and from neurons may be due to the fact that all peptide containing cells are embryologically related and therefore provided with similar membrane properties

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Structural and functional ontogenetic development of the rat portal vein after neonatal 6-Hydroxydopamine treatment

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The importance of the adrenergic vasomotor nerve supply for the vascular ontogenetic development has been studied in the isolated portal vein preparation from rats, at 5–6 week of age who had either been chemically sympathectomized by a series of postnatal 6-hydroxydopamine (6-OHDA) injections or been receiving the solvent alone. It was found that 6-OHDA treatment largely but not completely prevented the outgrowth of the terminal NA fluorescent ground plexus. Nevertheless, the media underwent seemingly normal differentiation into two layers. Functionally the portal vein from the 6-OHDA treated animals displayed weak and non-persistent myogenic spontaneous activity sensitivity to exogenous noradrenaline (NA) was increased 3-fold and maximum stress was increased by 25% as compared to control Responses to transmural field stimulation were only obtained at high impulse rates and the maximum response was attenuated Considering the very sparse adrenergic innervation following 6-OHDA they seemed surprisingly large however but since they were abolished by tetrodotoxin and by phenoxybenzamine responses are concluded to be neurogenic and adrenergic in origin. A singular attenuation of neurogenic responses by atropine was found in 6-OHDA treated vessels but not in controls. It is concluded that the adrenergic vasomotor nerve supply seems to exert some trophic influence during ontogenetic development but that the morphologic vascular development is largely governed by other non-neurogenic mechanisms. As to functional development, 6-OHDA induced sympathectomy causes impaired development of phasic myogenic activity whereas maximum stress is augmented as is the tissue sensitivity to exogenous NA

Key words Adrenergic vasomotor nerves, rats, ontogenesis, portal vein, trophic effects, vascular neuroeffector 6-OHDA

In most blood vessels the terminal adrenergic vasomotor nerve supply does not reach the individual smooth muscle cells but is confined to a two-dimensional plexus located at the adventitiomedial junction. Furthermore there is indirect evidence that in the isolated rat portal vein (see Ljung 1976) and in at least some arterial resistance vessels (see Bellfrage 1978), the α-receptor sensitivity also to exogenous noradrenaline (NA) is primarily located to the smooth muscle cells in close contact with the adrenergic nerve terminals The β-adrenoceptor mediated, inhibitory vasodilating influence however seems to be elicited throughout the media indicating a generalized β-adrenoceptor distribution (Belfrage 1978)

The media of the adult rat portal vein is divided into an outer longitudinal and an inner circular layer of smooth muscle The adrenergic ground plexus is located between these layers (Johansson et al. 1970) At birth the vessel wall consists of undifferentiated cells and the adrenergic nerves are restricted to bundles of nonterminal axons in the adventitia. During the first few weeks of life the media becomes differentiated and oriented into the two muscle layers. The nerve terminals penetrate the outer longitudinal layer and ramify on its inside to

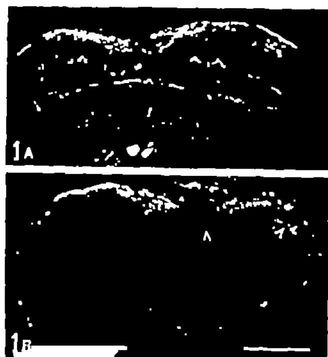


Fig 1 Cross-sections of portal veins from (A) a control rat and (B) a 6-OHDA treated rat Both rats were 5 weeks of age and pretreated with NA (5 μg/kg i v) 30 min before sacrifice to increase the fluorescense of adrenergic terminals Note that division of the media into a heavy outer (longitudinal) muscle layer and an inner (circular) layer The most pronounced terminal plexus can be seen on the inner aspect of the terminal layer in A after 6-OHDA treatment very few adrenergic fibres (A) are seen between the muscle layers. Fluorescence micrograph bar indicates 40 μm

form a plexus between the two media layers (Lundberg et al 1976) Correlated to this morphologic development a dramatic functional development occurs (Ljung & Stage 1975) The corresponding stages of ontogenesis of the portal vein occur prenatally in species more mature at birth than the rat (Stage & Ljung 1978)

Considering the asymmetrical vasomotor nerve supply and the differentiated adrenoceptor distribution in at least some sections of the vasculature it is of interest to explore the role of the adrenergic vasomotor nerve supply in the ontogenetic development of the vascular effector system In the present experiments the portal vein of rats chemically sympathectomized by intense postnatal 6-hydroxydopamine (6-OHDA) treatment (cf Provost, Bohus & de Jong 1974) has been studied and compared to control

METHODS

Rats of the Sprague-Dawley strain were mated in the laboratory in sufficient number to ascertain that at least two litters would later be delivered on the same day Each mother rat nursed eight baby rats Alternate litters received a series of subcutaneous 6-OHDA injections on the 1st nd (100 μg/g) 7th 14th, 20th and 26th (250 μg/g) postnatal day Control litters received s.c injections of vehicle alone (saline and ascorbic acid 200 μg/ml).

At the time of the expt during the fifth or sixth week of life one control rat and one 6-OHDA treated rat, born on the same day ("matched") were sacrificed by cervical fracture and the isolated portal veins were either studied directly by histological or histochemical methods (see below) or mounted in an organ bath for isometric recording of contractile activity and thereafter examined morphologically

Organ bath experiments The two portal veins from the matched animals were mounted in the same organ bath containing 30 ml of Krebs solution. One end of each tissue was tied to a tissue holder and the other end to a force transducer (Grass FT 0.03) under 2 mN passive force which was obtained by stretching the vessel to its approximate in vivo length The tissues were allowed to accommodate in the bath for 1 h before the experiment was started

The active force was recorded on a Grass polygraph and the mean active force of the phasic activity was obtained by electronic integration of the force signal over 1 min periods. The integrator output was recorded on a separate channel of the polygraph Responses to exogenous agents were induced by injections directly into the bath fluid in volumes of 0.3–0 9 ml Concentration–effect relationships for exogenously administered noradrenaline (NA) were obtained by sequential NA administrations at min intervals The cumulated NA concentrations were: 3 10, 30 nM 3 10 30 μM Neurogenic responses were elicited

Table 1 *Effects of postnatal 6-OHDA treatment on body weight left ventricular weight and cross sectional area of portal vein measured at 6 weeks of age (mean ± S E) (n=5)*

	Control	6-OHDA	*P*
Body weight (g)	121±5	90±4	0.001
Left ventricular weight (g)	0 35±0 016	0.29±0.013	0 020
Cross sectional area, longitudinal smooth muscle of portal vein (mm²)	0 14±0 003	0 10±0 007	0.003

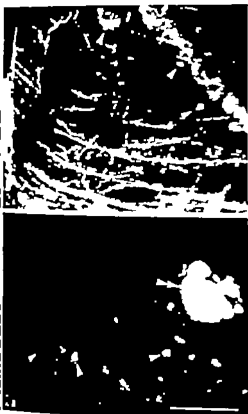


Fig. 2. Stretch preparations from (A) control rat and (B) 6-OHDA treated rat. Both rats were 5 weeks of age and the portal veins were mounted at the end of an organ bath experiment. Note the striking paucity of adrenergic nerve fibres after 6-OHDA treatment. The single remaining fibre, however shows clear varicosities. Mast cells indicated by ▷. The symbol ▷▷ indicates cluster of small strongly fluorescent cells sometimes observed in the adventitia of the portal vein. Fluorescent micrograph, bar indicates 40 μm.

by transmural field stimulation over 1 min periods by means of square wave pulses (0.8 ms, 15 V) at variable frequency. The impulses were delivered between platinum electrodes on either side of each preparation by Grass stimulator (model S4B) and the voltage was monitored on an oscilloscope.

Spontaneous myogenic contraction amplitude was determined as the maximum peak force recorded during 3 min period prior to the first ("early" phase of expt.) and the last ("late") response which was induced in particular expt. The amplitude was expressed in absolute (mN) as well as in relative terms (percentage of max force induced by NA). The latter in turn, was determined as the highest deflection of the force recording obtained during the cumulative NA exposure. In all other respects the magnitude of induced responses were quantitated as the mean force recorded during the period of exposure minus the mean force developed during the 3 min period of spontaneous activity immediately prior to stimulation. NA concentration–effect relationships were obtained by determining the least squares fit of such values from each expt. to tree hyperbolic function. ED_{50} values were determined from the these functions and utilized in logarithmic form ($-\log ED_{50}$(M)) in statistical calculations.

Fluorescence histochemistry. Portal veins for histochemistry were dissected from 5–6 weeks old rats, killed by decapitation. The vessels were either ( ) opened and stretched on glass slide as described previously (Lundberg et al. 1976) or (*b*) frozen (together with some splenic tissue for support) in liquid propane cooled by liquid nitrogen. One series of tissues were mounted according to ( ) at the end of organ bath expts. After drying over Siccapent ( ) or freezedrying in Hetosic® (model Thieme) freeze dryer (*b*) the specimens were reacted with paraformaldehyde vapour for 1 h at 80°C as described previously (.g. Corrodi & Jonsson 1967). After embedding in paraffine the freezedried specimens were sectioned in 10 μm thick sections and mounted in Entellane® with some xylene. All specimens were examined and photographed in Zeiss Junior fluorescence microscope for transillumination.

Some vessels were—prior to freezing—incubated in Krebs solution with Evans blue (10 μg/ml) in order to decrease the disturbing autofluorescence of elastin in the vessels (De la Lande & Waterson 1968). By this treatment the green autofluorescence of the elastin fibres is changed into bright red fluorescence, leaving the CA fibres green fluorescent as usual.

T improve the visibility of possibly weakly fluorescent CA fibres in 6-OHDA treated vessels, some animals were given NA i. (50 μg/kg) 30 min before killing. This treatment did not alter the fluorescence microscopical picture of these vessels.

Effector tissue cross sectional area. I 5 expts. the tissues were repeatedly rinsed in Krebs solution over a 30 min period after the last exposure to NA to allow for return of control activity. The organ bath was then lowered and beaker filled with Bouin solution was brought into position for over night fixation at room temperature. The tissues were then dehydrated, embedded, sectioned at 4 μm and stained according to routine procedures. Micrographs (18 24 cm) of the total vascular circumference were then obtained of the 10 vessels and of calibration scale. After coding, the examiner identified the longitudinal smooth muscle media layer cut it out from the photograph, weighed it and calculated the cross sectional area by making reference to the weight of the micrograph of 1 mm² square obtained in the same way. The code was then deciphered and the average cross-sectional area of the effector tissues and the average maximum stress induced by NA (mN/mm²) was determined for the two groups of tissues.

Experimental protocol. Two separate sets of experiments involving functional studies were performed.

Following the 1 h incubation period in the first set where 6 pairs of preparations were studied, the vessels were exposed to transmural field stimulation at 1 4 16 and 64 Hz at 15 min intervals. A cumulative NA dose-response curve was then determined. After a 30 min rinse

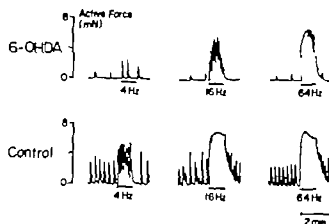


Fig 3 Tracings of spontaneous activity and responses to transmural nerve stimulation at graded frequencies of portal veins from rats 5 weeks of age either treated during the neonatal period with 6-OHDA (top) or with vehicle alone (bottom)

period transmural nerve stimulation (NS) at 16 Hz was applied The tissues were then exposed to the following sequence at 15 min intervals Acetylcholine (ACh) 10 μM 1 min Atropine 1 μM 30 min ACh 10 μM 1 min NS 16 Hz 1 min Tetrodotoxin 1 μM 15 min NS 16 Hz 1 min The tissues were then removed and quickly mounted as stretch preparations on glass slides for histochemical processing as described above

In the other set of expts 5 pairs of portal veins underwent a cumulative dose response study before they were rinsed over 30 min in normal Krebs solution NA in a concentration of 10 μM was then administered and once a plateau was reached $BaCl_2$ (5 mM) was added to obtain a true maximal contractile response On average the combination of NA and Ba^{2+} did not produce any greater response than did NA alone in the preceding series of NA exposures Therefore the peak values of that combination will not be presented in the result section Following a 30 min recovery period the tissues were fixed and processed for cross sectional area determination as described above

Solutions and drugs The Krebs solution had the following composition in mM NaCl 122, KCl 4.73 $NaHCO_3$ 15 5 KH_2PO_4 1 19 $MgCl_2$ 1 19 glucose 11.5 $CaCl_2$ and CaNa versenate 0 026 The solution was continuously bubbled with 4% CO_2 in O_2 and kept at 38°C

The drugs used were 1 noradrenaline bitartrate (l arterenol Sigma Chemical Co) acetylcholine chloride (Merck) phenoxybenzamine HCl (Dibenzyline® SK & F) Tetrodotoxine (Sigma) 6-hydroxydopamine (H [illegible] AB Hässle)

Statistics Results are presented as mean ± S E Statistical significance has been tested by Student's t-test (pairing design where appropriate) and has been considered probable for $P < 0\ 05$

RESULTS

Somatic development The intense postnatal 6-OHDA treatment seemed to retard the general development of the young rats as evidenced by a few days delay of the appearance of fur and of the opening of the eyes At the time of the expt i e at the age of 5–6 weeks the 6-OHDA treated animals displayed ptosis Their body weight (83±4 g mean ± S E n=6) was 21±4% less than that of their matched controls (104±6 g P<0 001 paired t test) In a subgroup of animals left ventricular weight was determined As seen in Table 1 left ventricular weight was reduced to approximately the same relative extent as body mass

Morphologic development of portal vein At five weeks of age the media of the portal vein is divided into an outer longitudinal muscle layer and an inner circular one (Fig 1 A) like in adult animals The same division was found in vessels from 6-OHDA treated rats (Fig. 1 B) The cross sectional area of the longitudinal muscle layer was determined in 5 pairs of vessels (Table 1) A reduced wall thickness was observed in the 6-OHDA treated group

Table 2 *Spontaneous force developed immediately prior to first stimulation ("early") and before last stimulation ("late") during the expt*

Note that the spontaneous myogenic activity was weak after neonatal 6-OHDA treatment both in absolute and relative terms and tended to fade away in the course of the expt (Mean ± S E n=11)

	Spontaneous activity				
	Control		6-OHDA		
Experimental period	mN	Per cent max NA	mN	Per cent max NA	P (mN)
Early	3 4±0 30	38±2 8	2 0±0 43	19±3.3	0 013
Late	3.2±0 45	3 ±4 4	1 2±0 60	2±2.0	0.014
P	0 66		<0 001		

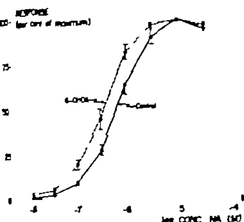


Fig. 4 Concentration–effect curves for portal veins from rats either treated with 6-OHDA during the neonatal period or with vehicle alone ("control"). Note slight shift to the left of curve for 6-OHDA exposed group (Mean ± S.E., n 11)

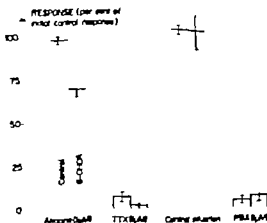


Fig. 6 Effects of various pharmacological interventions on effector responses to transmural field stimulation at 16 Hz. Portal veins from rats treated with 6-OHDA during the neonatal period or with vehicle alone ("control"). Note elimination of responses after tetrodotoxin (TTX) or phenoxybenzamine (PBA) (Mean ± S.E., n=6.)

In the control animals the terminal adrenergic structures were typically found between the two muscle layers, i.e. at the inner aspect of the longitudinal layer (Fig. 1 A) Predominantly nonterminal NA-fluorescent structures and strongly fluorescent chromaffine cells were present in the adventitia, i.e. on the outside of the longitudinal media layer In the portal veins repeatedly exposed to

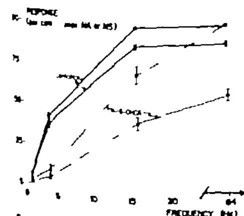


Fig. 5 Frequency–response relationships for transmural field stimulation of portal veins from rats either treated with 6-OHDA during the neonatal period or with vehicle alone ("control") Responses, on ordinate, either expressed in per cent of maximum response to NA (open circles) or as per cent of maximum response to transmural stimulation (filled circles). Note lack of significant response at 4 Hz. (Mean ± S.E., n=6.)

6-OHDA (Fig. 1B) a very marked reduction of specific NA fluorescence was observed and merely occasional terminals were noted in the cross sections. As described in Methods NA was administered to the rats before sacrifice in order to enhance neuronal fluorescence in cross-sectional preparations. In stretch preparations of portal veins from control animals (Fig. 2 A) a mesh of primarily terminal fibres are observed which thus displays the density of the essentially two-dimensional plexus located between the two media layers (Fig. 1 A) After 6-OHDA treatment only single terminal fibres (Fig. 2B) were found in the stretch preparations studied.

Functional development of portal vein. During the periods of control activity shown in the tracings of Fig. 3 the phasic spontaneous activity typical of the adult portal vein is apparent both in untreated and in 6-OHDA treated portal veins from rats of five weeks of age In the vessels from 6-OHDA treated animals however the amplitude of the spontaneous activity both in absolute terms (mN) and relative to the maximum force developed in response to exogenous NA (see below) was significantly lower at the beginning of the expt. and it actually faded away in the course of the expt. (Table 2) In tissues from untreated animals no significant decline in spontaneous force was observed during the organ bath expt.

The concentration–effect relationship for exoge

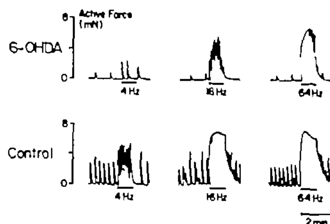


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Morphologic development of portal vein At four weeks of age the media of the portal vein is divided into an outer longitudinal muscle layer and an inner circular one (Fig 1 A) like in adult animals The same division was found in vessels from 6-OHDA treated rats (Fig 1 B) The cross sectional area of the longitudinal muscle layer was determined in 5 pairs of vessels (Table 1) A reduced wall thickness was observed in the 6-OHDA treated group.

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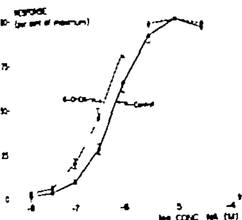


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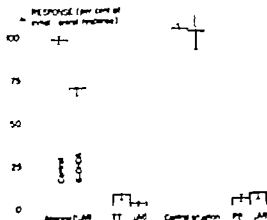


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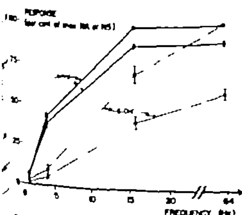


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Functional development of portal vein. During the periods of control activity shown in the tracings of Fig. 3 the phasic spontaneous activity typical of the adult portal vein is apparent both in untreated and in 6-OHDA treated portal veins from rats of five weeks of age. In the vessels from 6-OHDA treated animals, however, the amplitude of the spontaneous activity both in absolute terms (mN) and relative to the maximum force developed in response to exogenous NA (see below) was significantly lower at the beginning of the expt. and it actually faded away in the course of the expt. (Table 2). In tissues from untreated animals no significant decline in spontaneous force was observed during the organ bath expt.

The concentration–effect relationship for exoge

nous NA (Fig 4) was slightly displaced to the left for tissues from animals exposed to 6-OHDA The log ED_{50} value of these preparations was -6.38 ± 0.07 M (mean ±S E $n=11$) whereas it was -6.03 ± 0.07 M for the controls ($P=0.00?$)

The maximum force developed in response to NA did not differ 10 3±0 6 mN vs 9 0±0 5 mN ($P=0.11$) in the treated tissues as compared to control However since the muscle mass of the longitudinal media layer was decreased (Table 1) the maximum stress (force/area) was significantly elevated in the 6-OHDA treated group 103±9 mN/mm² compared to control 75±4 mN/mm² ($n=5$) It is thus evident that the functional properties of the effector tissue are indeed altered by postnatal 6-OHDA injections since spontaneous myogenic activity is altered and maximal NA response is augmented

Responses to transmural field stimulation could be elicited in all portal veins studied In those exposed to 6-OHDA however very high impulse rates were required for significant responses to occur (Figs 3 5) The frequency–response relationship (Fig 5) was markedly affected whether expressed as a percentage of the maximum responses to NS or to NA

In view of the morphological findings in the stretch preparations (see above) which were obtained in the very same tissues as studied in the organ bath experiments (see "Methods) it was surprising that stimulation of the extremely sparse nerve supply could at all mediate any detectable response The pharmacological analysis presented in Fig 6 indicates that this was indeed the case The response of each tissue to NS at 16 Hz was set at 100% Following atropine (1 μM) which completely blocked a postjunctional response to ACh (10 μM) the NS effect was unaltered in the control preparations and was reduced by 40% in the 6-OHDA treated ones Tetrodotoxin practically abolished the NS response an effect which was completely reversible Finally phenoxybenzamine (1 μM) was given and this again eliminated the response to NS at 16 Hz.

DISCUSSION

During the first 4 postnatal weeks the main steps occur of the dynamic ontogenetic development of the rat portal vein After that period quantitative rather than qualitative differences in the neuroeffector function are found when portal veins from young rats are compared to those of adults (Ljung & Stage 1975 Lundberg et al 1976) In the present expts we chose to study the structural and functional properties of the vessel during the fifth and sixth weeks of life since it appeared that any interference with normal development caused by repeated 6-OHDA exposures would most likely be detectable at this time

It has previously been shown that injections of 6-OHDA during the postnatal period in rats lead to inefficient vasomotor nerve control in the adult animal due to permanent sympathectomy (see Clark Laverty & Phelon 1972 Provost et al. 1974). In spite of intense treatment in the present experiments a few terminal nerve fibres were detected in the portal vein preparations at the typical location, i e between the longitudinal and the circular smooth muscle layers of the media It is possible that these fibres had escaped destruction either because they had not been exposed to 6-OHDA in sufficiently high local concentrations after all, or because they were incapable of neuronal uptake of 6-OHDA It was obvious however that extremely few adrenergic terminal nerve fibres did exist in the portal veins which had been exposed to 6-OHDA. In preliminary expts we observed that such paucity of adrenergic innervation prevailed throughout the first four postnatal weeks It seems most likely that the 6-OHDA treatment primarily prevented outgrowth and maturation of the terminal axons into the vascular wall rather than causing the nerve damage after functioning nerve—smooth muscle contacts had been established

It is remarkable that in spite of the very considerable reduction in fluorescent adrenergic nerve terminals in the portal veins from the 6-OHDA treated group of rats responses to transmural field stimulation could be elicited Actually the maximum response amounted to more than 50% of that to exogenous NA However it was obvious that the frequency–response relationship was markedly displaced to the right i e very high impulse rates were required to elicit a response In fact such high impulse rates hardly occur in vasomotor nerves for any long periods of time (Folkow 1952 Little Wennergren & Öberg 1975) and we conclude that in the portal veins of the 6-OHDA treated rats no functional excitatory control had been operating during the postnatal period The apparent discrepancy between the few remaining ...ergic terminals and

the magnitude of the effector response to transmural field stimulation prompted the pharmacological analysis of the transmitter function presented in Fig. 6. It was found that the responses were abolished by tetrodotoxin as well as by phenoxybenzamine in a moderate concentration. We therefore conclude that the responses were due to selective activation of intramural adrenergic nerves. Apparently myogenic propagation caused spread of excitation from the areas directly activated by the released transmitter to the remainder of the media (Johansson & Ljung 1967, 1968). Atropine was also included in the pharmacological analysis because findings from other tissues indicate that cholinergic nerves might develop after adrenergic denervation (cf Koslow et al. 1977). The response to transmural stimulation was indeed transiently reduced in the presence of atropine. The evaluation of this observation will require further analysis.

In spite of the paucity of vasomotor nerve supply after 6-OHDA treatment the media of the portal vein had clearly become divided into an outer longitudinal and an inner circular muscle layer like in the control tissues. In the rat this division and effector cell orientation begins late during the first postnatal week (Ts ao et al. 1971, Lundberg et al. 1976). Determination of smooth muscle cross sectional area indicated that the effector cell mass was decreased in the 6-OHDA exposed group. It has previously been found (Sutter & Ljung 1977) that surgical denervation by bilateral coeliac ganglionectomy leads to increased cross sectional area of the adult rat portal vein. It may be that selective removal of the splanchnic vasomotor nerve supply causes elevated transmural pressure in the portal vein which in turn is known to cause vascular wall hypertrophy (Johansson 1976). Conversely, postnatal 6-OHDA treatment causes reduced blood pressure (Provost et al. 1974) and it appears likely that the transmural pressure in the portal vein is not elevated following such generalized sympathectomy. Rather the 20–30% reduction in smooth muscle mass found in the portal vein may be interpreted to reflect the general retardation in growth evidenced by body weight and left ventricular weight which were reduced to the same relative extent. From the morphological point of view it is concluded that elimination of the substantial part of vasomotor nerve development by neonatal 6-OHDA treatment caused only minor changes in the ontogenesis of the vascular effector.

The most conspicuous functional consequence of the 6-OHDA regime was of course the attenuation of responses to nerve stimulation of the isolated portal vein as discussed above. It was quite obvious, however, that several other functional properties were also altered in these tissues when studied in vitro. The spontaneous myogenic activity characteristic of smooth muscle of the adult portal vein, normally displays peak force amplitudes of around 50% of those of the maximum force amplitude in response to optimal NA concentrations. During the fifth week of life the corresponding value is 30–40% (Table [illegible], Ljung & Stage 1975) under control conditions. 6-OHDA treatment led to much weaker phasic activity (Table [illegible]) which furthermore tended to disappear during the experiment. In portal veins of adult animals surgical denervation does not cause any apparent interference with spontaneous myogenic activity (Johansson et al. 1970). Aprigliano & Hermsmeyer (1977) found that 6-OHDA treatment of adult rats resulted in postjunctional changes in the properties of the portal vein smooth muscle. There was a slight decrease in resting membrane potential which tended to return towards the control level within 5 days after treatment. The consequence for the mechanical spontaneous force development were not commented upon by the authors. It is likely that the alterations observed in the vascular tissue following 6-OHDA treatment at an adult stage are qualitatively different from those which manifest themselves as reduced spontaneous activity in the neonatally denervated portal vein (Fig. 4, Table 2), i.e. a situation where the smooth muscle has developed in the absence of sympathetic vasomotor nerve control. Schematically the sympathetic recorded spontaneous activity reflects inherent pace-maker activity and efficient myogenic spread of excitation within the longitudinal muscle (cf Johansson & Ljung 1967, Ljung & Stage 1970). The finding of low rate and low relative contraction amplitude of the spontaneous activity in 6-OHDA exposed portal veins might indicate increased membrane potential stability. This is compatible with an overall hyperpolarization of the 6-OHDA treated tissues as compared to control.

As to responses to exogenous NA, increased sensitivity to the agent (expressed as log ED_{50} value) and increased maximum stress were found. The shift of the NA concentration effect curve (Fig. 3) is less than that found after surgical denervation or

pharmacological inhibition of the neuronal NA uptake mechanism in the adult rat portal vein (Johansson et al 1970 Ljung Bevan & Su 1973) Aprigliano & Hermsmeyer (1977) did not find pre junctional supersensitivity of any marked degree after 6-OHDA in the adult rat portal vein It is not known to what degree of prejunctional NA super sensitivity complete elimination of neural NA uptake would lead in portal veins at the age of five weeks One interpretation of the present findings of limited NA supersensitivity after 6-OHDA treat ment however is that the neuronal uptake of the few remaining adrenergic terminal neurons would be sufficient to reduce the apparent NA sensitivity Conversely this interpretation albeit speculative would imply that following 6-OHDA treatment dur ing the neonatal period the α-adrenoceptors on those few smooth muscle elements in close contact with the few existing terminal axons would primarily determine the response also to exogenous NA (cf Johansson et al 1970 1972 Ljung et al 1973) This speculation would imply that the existence of a functioning nerve supply to a smooth muscle cell in the rat portal vein would determine the α-receptor population and thus the sensitivity to NA rather than the possibility that α receptor supply of par ticular effector cells would direct the outgrowth of the adrenergic nerve terminal

In conclusion the present expts on the effect of intense neonatal 6-OHDA treatment of the rat indicate that outgrowth of a vasomotor nerve supply is interfered with to a great extent but not completely prevented In spite of an apparent elimination of a functioning adrenergic innervation differentiation and development of the media occurred It appears that the scarce nerve supply is instrumental in determining some aspects of the effector function but that ontogenesis may largely be determined by other factors than the adrenergic nervous system

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Dynamics of beta adrenoceptor induced amylase release and cyclic AMP accumulation in the guinea pig submandibular gland

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The dynamics of amylase release from the guinea pig submandibular gland were studied in vitro by applying a multi-channel microperifusion set. This technique makes it possible to measure time related enzyme release more accurately and to take samples of perifused tissue at short intervals. Stimulation of the β-adrenoceptor with norepinephrine gives rise to a rapid initial enzyme discharge detectable within 15 s. Administration of propranolol inhibits enzyme release which is not restored after removal of the agent. Simultaneous measurements of tissue cyclic AMP during norepinephrine stimulation at various time intervals display a significant increase of cAMP as early as 15 s after stimulation of secretion. This increase of cAMP thus coincides with the discharge of amylase. In addition, cAMP continuously accumulates during 30 min of norepinephrine perifusion of the slices. The present study describes a valuable tool with high sensitivity for visualizing the relations between enzyme secretion from the salivary gland and the intracellular biochemical processes. The data obtained further indicate a close correlation between amylase and cAMP during the initial phase of enzyme discharge

Key words Amylase secretion, adenylate cyclase cyclic AMP perifusion, salivary glands

Catecholamines stimulate adenylate cyclase activity in different tissues resulting in increased levels of intracellular cAMP (Sutherland & Robison 1967) In the rat parotid gland enzyme discharge is induced by catecholamine interaction with β-adrenoceptors and it has been assumed that parotid amylase release is mediated by changes in the intracellular concentrations of cAMP (Bdolah & Schramm 1965) The plasma membranes of the rat parotid acinar cells are supplied with adenylate cyclase which is activated by e.g. norepinephrine (Schramm & Naim 1970 and the activation of the enzyme causes a marked accumulation of cAMP (Batzri et al. 1973) It has been suggested that the β-adrenoceptor of rat parotid gland is mainly of β -type (Butcher et al. 1975, Carlsöö et al. 1978), similar to the β-receptors in heart and adipose tissue However stimulation of amylase release by adrenergic agonists without detectable increases of intracellular cAMP accumulation has also recently been described (Butcher et al. 1975)

In guinea pig submandibular gland amylase and peroxidase secretion can be induced by both DBcAMP as well as theophylline a phosphodiesterase inhibitor (Carlsöö et al. 1974) Furthermore the catecholamines activate the adenylate cyclase system in this gland (Bhoola & Lemon 1973 1975)

To elucidate the role of cAMP in stimulus-secretion coupling of the salivary gland the dynamics of amylase release and its relation in time to changes in levels of cAMP has to be established. In the present study a multi-channel microperifusion system (Idahl 1972) was employed which makes it possible to obtain concomitant measurements of amylase secretion and intracellular cAMP during short periods of perifusion.

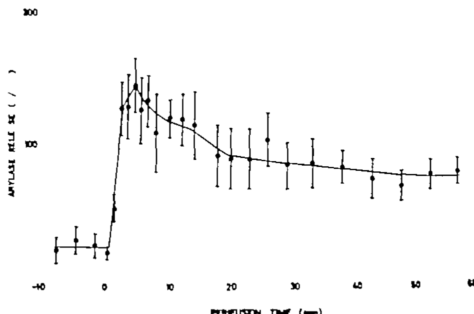


Fig 1 Dynamics of amylase release from perifused guinea pig submandibular gland in response to norepinephrine The horizontal bar denotes perifusion with medium containing the stimulator Three pieces of glandular tissue were perifused in three parallel chambers and the effluents from these chambers were pooled Continuous sampling began 6 min after the perifusion was started and the samples were collected as indicated over 1 2 3 or 5 min periods The points represent mean values±S E. during each sampling period with the points located at the center of the sampling periods. Zero time marks the change of medium no correction was made for the dead space of the perifusion system Amylase release into the perifusion medium was expressed as U/min One unit of amylase activity is defined as the amount of enzyme that liberates 1 μmol maltose per min at Ph 6 9 and 25°C Five separate expts. were performed.

MATERIALS AND METHODS

Animals Male guinea pigs 3 to 4 months old were fasted overnight. The submandibular glands were rapidly removed under sodium pento-barbital anesthesia, then immersed in basal medium at 20°C and carefully freed from extraglandular tissue Pieces of uniform size (length 3 mm width 0 7–1 0 mm) were excised from the glands under a stereo microscope Care was taken to avoid adipose tissue larger vessels and ducts as well as masses of connective tissue in the glands

Perifusion A non-recycling multi-channel perifusion system was used (Idahl 1972) This micro system was modified to allow perifusion with control and test media fed to different tissue chambers which were inserted parallel to one another For this purpose the incubation media were led from a modified sliding valve beneath the reservoirs via two separate tubes (0.5 mm i.d) feeding two identical manifolds each of which suppl ed 5 tissue chambers

Three to ten excised glandular pieces were transferred by means of a braking pipette to an equal number of perifusion chambers (volume 4.5 μl) The tissue pieces were then perifused with gassed (95% O_2 5% CO_2) medium at a rate of 17 μl per min and chamber Perifusion pressure (1.5–2 0 kPa) (1 kPa=7.5 mmHg) and oxygen partial pressure (75–90 kPa) were continuously monitored at the tissue chamber level during the experiment At the above perifusion rate it took 60 s for the medium to pass from the reservoir to the tissue chamber and another 30 s to reach the effluent outlet. Furthermore due to the elongated shape of the tissue chamber and the very narrow tubing in the crucial parts of the system the time for a complete change of medium in the system was only 90 s. Sampling began 6 min after the perifusion was started Samples of effluent were taken continuously at intervals indicated in the figures A prestimulatory period of 15 min with basal medium preceded the change to medium containing norepinephrine in order to equilibrate the tissues. Amylase activity in the samples was assayed by a micromodification of the dinitrosalicylate method as described elsewhere (Danielsson 1974)

In experiments designed to measure the tissue content of cyclic AMP perifusion chambers containing tissue pieces were d sconnected and plunged into melting pentane at the intervals indicated in the figures. The chambers were then dismounted at −25°C the inner tube containing the frozen tissue were transferred to a vacuum tube and freeze-dried (0 1 Pa, −30°C) The dry tissue pieces were then weighed on an ultramicro-balance (L 7 Mettler Instruments AG Zurich Switzerland) Cyclic AMP was extracted and assayed radioimmunologically as described by Hellman et al (1974)

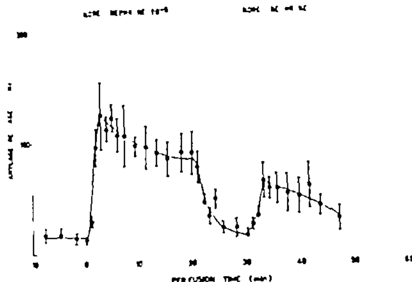


Fig. 2. Effect of discontinuous stimulation with norepinephrine on amylase release from perfused guinea pig submandibular gland. The experimental design was as described in the legend to Fig. 1. However, stimulation with norepinephrine was interrupted for 10 min as indicated by the bars. Amylase was sampled and the result expressed as in Fig. 1. Five separate expts. were performed.

Chemicals and incubation medium. L-Norepinephrine bitartrate came from Sigma Chemical Co., St. Louis, Mo., USA and L-propranolol was a gift from ICI Pharma AB, Göteborg, Sweden. ^{125}I-labelled succinyl cyclic AMP tyrosine methyl ester and cyclic AMP antibodies were bought from Schwarz/Mann, Orangeburg, N.Y., USA. Bovine serum albumin (fraction V) was obtained from British Drug Houses Ltd., Poole, England. Other reagents were of analytical grade; deionized sterile filtrated water was used throughout.

Krebs-Ringer Henseleit bicarbonate buffer (Krebs [illegible]) containing serum albumin (1 g/l) and glucose ([illegible]) was used as the basal incubation medium.

RESULTS

Fig. 1 illustrates the dynamics of amylase release from minute pieces of submandibular gland as measured in the multi-channel perifusion system. Stimulation of secretion with continuously delivered norepinephrine gives rise to an initial rapid peak of amylase, which thereafter slowly declines. At 60 min of perfusion time there is still a considerable amount of amylase secretion. No signs of double peak release are detectable. Discontinuation of norepinephrine stimulation rapidly reduces amylase secretion to basal level (Fig. 2). When readministered a second peak of amylase is obtained although lower than that initially recorded. On the other hand when instead of excluding perfusate which contains norepinephrine L-propranolol (a non-selective β-adrenoceptor blocking agent) is used the inhibition of secretion is delayed. The dose of L-propranolol employed in the experiments illustrated was equimolar to that of norepinephrine. After removing the β-blocker the expected second peak of amylase release did not appear indicating a high affinity of the drug to the receptor (Fig. 3).

Time dependent relations between norepinephrine-stimulated amylase secretion and accumulation of cAMP are depicted in Fig. 4. There were rapid increases in both amylase in medium and tissue cAMP. cAMP increases continuously during the period of observation despite the fall of amylase release. The total increase of cAMP amounted to 40–50 times the prestimulatory values. All experiments were performed in the absence of a phosphodiesterase inhibitor. Fig. 5 shows the early time course relationship between intracellular accumulation of cAMP and amylase secretion from the guinea pig submandibular slices in response to norepinephrine. As early as at 15 s when the first samples were taken both amylase and cAMP had

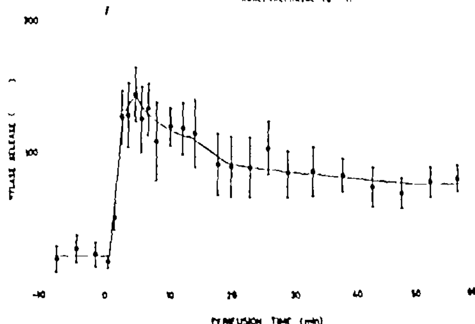


Fig. 1. Dynamics of amylase release from perifused guinea pig submandibular gland in response to norepinephrine. The horizontal bar denotes perifusion with medium containing the stimulator. Three pieces of glandular tissue were perifused in three parallel chambers and the effluent from these chambers were pooled. Continuous sampling began 6 min after the perifusion was started and the samples were collected as indicated over 1, 2, 3 or 5 min period. The points represent mean values ± S.E. during each sampling period with the points located at the center of the sampling period. Zero time marks the change of medium; no correction was made for the dead space of the perifusion system. Amylase release into the perifusion medium was expressed as U/min. One unit of amylase activity is defined as the amount of enzyme that liberates 1 μmol maltose per min at ph 6.9 and 25°C. Five separate expts. were performed.

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Perifusion. A non-recycling multi-channel perifusion system was used (Idahl 1972). This micro system was modified to allow perifusion with control and test media fed to different tissue chambers which were inserted parallel to one another. For this purpose the incubation media were led from a modified sliding valve beneath the reservoirs via two separate tubes (0.5 mm i.d.) feeding two identical manifolds, each of which supplied 5 tissue chambers.

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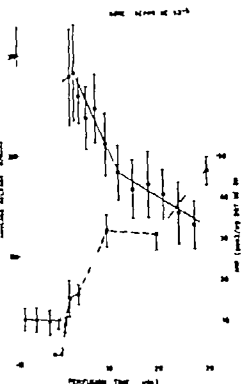


Fig. 4. Relation between amylase release and cAMP accumulation in perifused guinea pig submandibular gland during stimulation with norepinephrine. Nine pieces of tissue were placed in separate perifusion chambers and perifused parallel to one another. The chambers were consecutively disconnected and frozen during the perifusion at intervals indicated in the diagram for determination of the tissue content of cAMP. The pooled effluents from the two chambers, which were disconnected simultaneously at the end of the expt. (time 30 min) were sampled for assay of released amylase. Continuous sampling began 5 min after the perifusion was started and the samples were collected as indicated during 1, 2 or 3-min periods. The horizontal bar denotes perifusion with medium containing norepinephrine. The zero time indicates the change of medium. Whereas the points representing tissue content of cAMP are situated at the time of freezing, the points denoting the amylase release are located at the center of the sampling periods. Compensation for the dead space of the perifusion system was made in the diagram according to the observed time for the medium to pass from the valve to the tissue chambers and eventually to the sampling port. Amylase was expressed as in Fig. 1. Tissue content of cAMP was expressed as pmol/mg tissue dry weight. The points represent mean values ± S.E. for 5 separate expts.

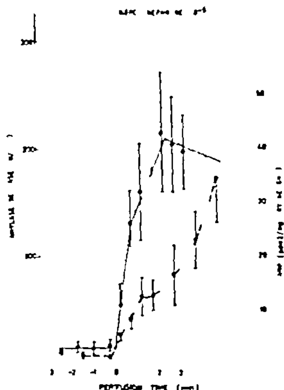


Fig. 5. Relation between amylase release and cAMP accumulation in guinea pig submandibular gland during the initial 5-min period of norepinephrine stimulation. The experimental design was as described in the legend to Fig. 4 except that 10 chambers with tissue were run in parallel and that the sampling intervals were shorter. Amylase release and tissue content of cAMP were expressed as in Fig. 4. Five expts. were performed.

by means of adrenergic agonists (Butcher et al. 1975).

To investigate further the time course relations between amylase release and changes in intracellular cAMP in salivary glands a sensitive perifusion system (Idahl 1972) was applied to minute isolated pieces of guinea pig submandibular gland. The multi-channel technique makes it possible to carry out tissue sampling at very short intervals.

Amylase was released at an increased rate as early as within 15 s after norepinephrine reached the tissue. At this time a marked increase in the cAMP levels was also recorded. Previous investigations on the cAMP levels in secreting salivary glands have shown a similar early increase in the cyclic nucleotide levels. Albano & coworkers (1976) recorded an apparent increase within as little as 5 s after norepinephrine stimulation of the guinea pig submandibular gland, and in the rat parotid Butcher et al. (1975) documented a rapid cAMP accumulation (within minutes) after isoprenaline and norepinephrine stimulation. In addition the lat

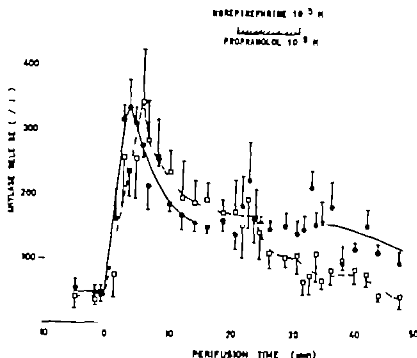


Fig 3 Effect of L-propranolol on norepinephrine induced amylase release from perifused quinea pig submandibular gland The design of the expts is as described in the legend to Fig. 1 except that six chambers containing glandular tissue were perifused in parallel Three of these chambers were exposed to norepinephrine (black bar) alone (●-●) The remaining chambers were fed L-propranolol (10⁻ M) for 10 min (hatched bar) during the period of norepinephrine (black bar) stimulation (□-□) Amylase was sampled from each set of three chambers and the results are expressed as in Fig 1 Five separate expts were performed

increased in equal proportions Thus the formation of cAMP is rapid and at least as fast as the first detectable enzyme discharge The initial phase of cAMP formation is almost linear and closely fol lows the curve of amylase release

DISCUSSION

Induction of secretion in exocrine cell systems in itiates a series of complex cellular events all of which are important for the final expulsion of sec retory material Several of these processes e g cAMP accumulation Ca K and Na fluxes as well as membrane depolarization have been studied in vivo and in different in vitro systems However it has often been difficult to establish what—if any—time relationship exists between the different processes involved Adenylate cyclase which is associated with cellular membranes, catalyzes the formation of intracellular cAMP (Sutherland & Robison 1967) the adenylate cyclase of the salivary gland cell membrane may be identical with the β-re ceptors (Yamamoto et al 1968 Robison et al 1967) *In vivo* studies of the rat parotid gland have established that adenylate cyclase activity in sali ary glands is increased by isoproterenol (Malamud 1972) and that a stimulation of adenylate cyclase in rat parotid glands is induced by norepinephrine process which is inhibited by L propranolol (β adrenoceptor blocking agent) (Selinger & Schramm 1971) In the guinea pig submandibular gland induc ti n of enzyme secretion by dibutyryl cAMP has been described (Carlsöö et al 1974) as well as catecholamine activated adenylate cyclase (Bhoola & Lemon 1973 1975 Albano 1976) Thus a sub stantial amount of data support the proposal by Schramm & coworkers (Schramm & Naim 197 Selinger & Schramm 1971) that the effect of β adrenergic agonists on amylase release from sali ary glands is mediated by changes in intracellular concentrations of cAMP However recently data have been presented which suggest that under certain conditions large increases in cAMP may not be necessary for stimulation of amylase release

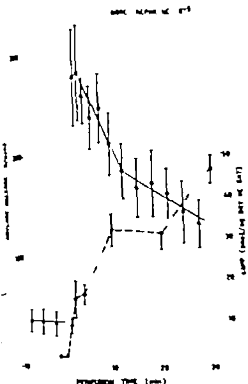


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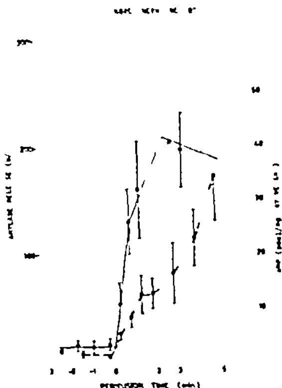


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To investigate further the time course relations between amylase release and changes in intracellular cAMP in salivary glands a sensitive perifusion system (Idahl 1972) was applied to minute isolated pieces of guinea pig submandibular gland. The multi-channel technique makes it possible to carry out tissue sampling at very short intervals.

Amylase was released at an increased rate as early as within 15 s after norepinephrine reached the tissue. At this time a marked increase in the cAMP levels was also recorded. Previous investigations on the cAMP levels in secreting salivary glands have shown a similar early increase in the cyclic nucleotide levels. Albano & coworkers (1976) recorded an apparent increase within as little as 5 s after norepinephrine stimulation of the guinea pig submandibular gland and in the rat parotid Butcher et al. (1975) documented a rapid cAMP accumulation (within minutes) after isoprenaline and norepinephrine stimulation. In addition the lat

ter authors detected a maximal level of cAMP after 5–10 min followed by a marked decline after 30 min of stimulation This is in contrast to the present study where significantly high levels of cAMP were also discernible after 30 min This discrepancy may be explained by the fact that in the present investigation the salivary gland pieces were continuously perifused and exposed to fresh medium containing the neurotransmittor Moreover Wojcik & coworkers (1975) have studied the effect of epinephrine on amylase release and cyclic nucleotide accumulation in the rat parotid gland in vitro The authors recorded a 6-fold increase in cyclic GMP levels at 5 min which declined markedly over the next 25 min The amine also stimulated a 5-fold increase in cyclic AMP levels which was sustained for at least 30 min

The sympathetic neurotransmittor norepinephrine employed as secretagogue in the present study has affinities to both α and β-adrenoceptors which control both enzyme and primary water and electrolyte secretion from salivary glands Potassium release as well as membrane hyperpolarization and resistance reduction is caused by α-adrenoceptor activation (Batzri et al 1973 Petersen & Pedersen 1974 Petersen 1976) whereas β-adrenoceptor activation causes no potassium discharge (Batzri et al 1973) By using a sensitive system the kinetics of epinephrine and/or norepinephrine induced potassium release from salivary glands can be analyzed and the time lag between hormone addition and ion release has been recorded as being no more than 2 to 3 s This time lag appears somewhat less than that observed for the onset of enzyme secretion (Batzri et al 1973 Schramm et al 1965) However with regard to enzyme release it is often difficult to determine exactly the time lapse between the addition of the secretagogue and the discharge of intracellular macromolecules into the medium With the present most sensitive perifusion system we were able to perform accurate determinations 15 s (the time required for perifusate sampling and freezing amounted to about 1 s) after the addition of norepinephrine At this time interval (15 s) a prompt amylase discharge was recorded as well as an accumulation of intracellular cAMP These findings that the effects of catecholamines on cyclic AMP coincide with the earliest detectable effect of α-amylase release are in accord with those reported by Batzri et al (1973) and Butcher et al (1975)

Amylase secretion from the guinea pig submandibular gland was found to cease almost immediately on removal of the stimulus, and a prompt second enzyme release response was observed when norepinephrine was once more included in the perifusate medium However this type of second response could not be detected after perifusion of the gland slices with l propranolol indicating a strong binding capacity of the drug to the β-adrenoceptor sites on the acinar cell membrane.

In conclusion the described multi-channel perifusion system originally designed for the study of insulin release from the pancreatic islet β-cells constitutes a valuable tool in the study of the dynamics of the secretory processes in salivary glands The effect of norepinephrine on the cAMP accumulation within the secretory cells was found to coincide with the earliest detectable effect on amylase discharge The results further support the available evidence for a second messenger role of cAMP in the process of enzyme secretion by salivary gland

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Formation and action of prostacyclin in the isolated human umbilical artery

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HAMBERG M TUVEMO T SVENSSON J & JONSSON C E Formation and action of prostacyclin in the isolated human umbilical artery Acta Physiol Scand 1979 106 289–292. Received 20 Dec 1978 ISSN 0001-6772 Department of Chemistry II and Plastic Surgery Karolinska Institute Stockholm, and Department of Paediatrics University Hospital Uppsala S eden.

The transformation of [1 ^{14}C]arachidonic acid by homogenates of human umbilical arteries s studied The major compound formed was the stable end product of PGI e 6-keto-PGF$_{1\alpha}$ (lactol form) as analyzed by gas-liquid hromatography-ma spectrometry PGI was generated by incubating PGH with lyophilized pig aorta microsome preparation PGI concentrations around 10 ng/ml rela ed the human umbilical artery preparation significantly Formation of PGI by umbilical arteries during pregnancy might be mechanism for regulation of blood flow to the fetus

Key ord Umbilical artery human, new born, prostacyclin prostaglandin, biosynthesis, in itro

During the last few years potent contractors of the human umbilical artery ha e been found among the prostaglandins (PGs) e g the PG endoperoxides PGG$_2$ and PGH$_2$ (Tu emo et al 1976) and thromboxane A$_2$ (TxA$_2$) (S ensson et al 1977) These short-lived compound are active at concentrations of 1 ng/ml and higher In earlier studies we have demonstrated the formation of some of the table end products of the short-lived compounds TxB$_2$ and PGE$_2$ in human umbilical arteries in amounts suggesting importance of the former products in the physiological closure of the arteries at birth (Tuvemo et al 1976, Jonsson et al 1976) In late 1976 Moncada and co-workers (Moncada et al 1976 Bunting et al. 1976) reported that rabbit and pig aortic microsomes transformed the PG endoperoxides to prostacyclin (PGI) a platelet aggregation inhibiting substance with relaxing effects on rabbit mesenteric and celiac arteries In this communication we report the formation of prostacyclin in human umbilical artery homogenates and the smooth muscle effects of prostacyclin on the isolated artery Preliminary data from this study were presented at the International symposium on Prostaglandins in neonatal medicine Toronto 1977 (Tuvemo 1978).

METHODS AND RESULTS

T a formation of a chidonic acid in huma mbilical artery homogenates

Human umbilical cords were taken immediately after delivery and transported in ice-cold Ringer medium The arteries were dissected free from remaining umbilical cord ti sue Arteries (3 g) and remaining umbilical cord tissue (3 g) were minced in 9 ml of ice-cold 0 1 M potassium phosphate buffer pH 7 4 and homogenized for ×60 s with a Potter Elvehjem type homogenizer [1 ^{14}C] arachidonic acid 12 µg (µCi purchased from The Radiochemical Centre Amersham) was dissolved in 50 µl of ethanol and added to each homogenate The homogenates were incubated with shaking at 37° for 15 min and then added to 5 vol of ethanol The diethyl ether extracts were treated with diazomethane and subjected to thin layer radiochromatography The solvent system used was the organic layer of ethyl acetate–... 4-trimethylpentane–water (50 100 100 / /) Three peaks of radioactivity appeared i.e Compound I (close to the solvent front; corresponding to arachidonic acid not converted) Compound II (slightly les polar than the reference i.e cholesterol cor

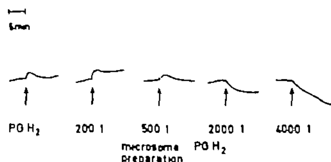


Fig 1 Effect of PGH (left) and incubation mixture of PGH and increasing amounts (200–4000 ng/ng PGH) of pig aortic microsome preparation (right) on human umbilical artery strip in organ bath experiment The final concentration of prostaglandin was 10 ng/ml

responding to monohydroxy acid(s)) and Compound III (close to the origin) Compound III was recovered from the silica gel and rechromatographed in a more polar solvent system i e methanol-diethyl ether (2 98 v/v) A major peak (20–40% of the recovered radioactivity (arterial homogenate) 3–7% of the recovered radioactivity (homogenate of the remaining umbilical cord tissue) appeared close to the reference the methyl ester of PGE_2 (R_f=0 39)

In another experiment 10 g of umbilical artery was homogenized and incubated with 190 μg of [1 C] arachidonic acid Compound III was isolated by preparative thin layer chromatography (yield about 25%) An aliquot was converted into the trimethylsilyl (TMSi) derivative by treatment with hexamethyldisilazane and trimethylchlorosilane in pyridine and analyzed by gas-liquid chromatography–mass spectrometry A single peak with a retention time corresponding to C 24 5 appeared (column 1% SE 30 on Gaschrom Q column temperature 240°) The mass spectrum showed ions of high intensity at m/e 510 (M) 420 (M 90 loss of CH (OTMSi)–TMSiOH) 337 (M 173 loss of CH (OTMSi)–$(CH_2)_4$–CH_3) 315 225 195 and 173 (CH (=O TMSi)–$(CH_2)_4$–CH_3) This mass spectrum was in complete accord with that obtained following gas-liquid chromatography of the methyl ester–TMSi derivative of the hemiketal form of 6-keto-$PGF_{1\alpha}$ (Johnson et al 1976) Another part of Compound III was treated with O-methyl hydroxyl amine hydrochloride in pyridine This afforded the O-methyl-oxime derivative of the methyl ester of 6-keto-$PGF_{1\alpha}$ as judged by thin layer radiochromatography and by gas liquid chromatographic–mass spectrometric analysis of the corresponding TMSi derivative (cf Pace Asciak 1976).

Finally aliquots of Compound III were treated with sodium borohydride in methanol and with 0.5 M NaOH in ethanol-water (1 1 v/v). This did not result in significant conversion as expected for the hemiketal form of 6-keto-$PGF_{1\alpha}$ (Pace Asciak 1976)

Prostacyclin action on human umbilical artery

Preparation of pig aortic microsomes A lyophilised microsome preparation from pig aortas was used according to Moncada et al To test the ability of the microsome preparation to form prostacyclin 5 mg of microsome powder was dissolved in 1 ml of 0 05 M Tris buffer pH 7 4 and incubated with 1 μg of PGH_2 at 22°C for 2 min An aliquot was then added to a sample of citrated platelet rich plasma Complete inhibition of aggregation induced by arachidonic acid (100 μg/ml) was found There was no inhibition if an aliquot of the microsome-P[illegible] incubate was tested in the same way after 30-[illegible] min This was taken as a criterion of formation of labile prostacyclin (Moncada et al 1976)

Organ bath experiments Spiral human umbilical artery strips were prepared from cords taken immediately after delivery as described earlier (Strandberg & Tuvemo 1975) and mounted for isotonic recordings in 5 ml organ baths containing Krebs-bicarbonate-glucose solution at pH 7.[illegible] temp 37°C pCO_2 40 mmHg and pO_2 100 mmHg Prostacyclin for these experiments was prepared b[illegible]

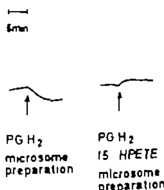


Fig 2 Inhibition of prostacyclin formation by 15-HPETE *Left* effect of incubation mixture of PGH and microsome preparation (ratio 1 4000 *Right* effect of incubation mixture of PGH and microsome preparation (ratio 1 40[illegible] containing 15-HPETE (1 μg/ml of incubation mixture The final concentration of prostaglandin was 10 ng/ml

incubating different amounts of the pig aortic microsome powder with PGH in Krebs solution at 37°C pH 7.4 for 90 s. Twenty-five to 100 µl of the incubates containing µl of acetone or less were introduced into the baths. Amounts of acetone less than 5 µl in the baths did not influence the tone of the preparations. To define an optimal relation between microsomes and endoperoxides fixed amounts of PGH with increasing amounts of microsome powder was used. Later a fixed ratio between these two were used (4 µg microsome/ng PGH_2) and the amounts of incubate varied.

Smooth muscle effects. Incubates containing PGH_2 and buffer with no microsome powder added produced contractions of the isolated artery. When greater amounts of the enzyme powder was used the addition of the incubates relaxed the artery preparations. –4 µg of the microsome preparation per ng PGH_2 seemed to produce maximal conversion of the contracting endoperoxide to the relaxing prostacyclin (Fig. 1). Assuming a complete conversion at these enzyme/substrate ratios 10 ng/ml of prostacyclin was necessary to produce a significant relaxation of the arteries. The relaxing effect of 10 ng/ml of prostacyclin was comparable to that of 40 ng/ml of PGE_1. When incubation was prolonged relaxing capacity was still found after 30 min and in some experiments after 60 min but not after 90 or 120 min.

15-hydroperoxy 5,8 11 13-eicosatetraenoic acid (15-HPETE) (Hamberg & Samuelsson 1967) 1 µg/ml, added to the incubate inhibited the formation of the relaxing compound (cf. Moncada et al 1976). In 4 expts. no relaxation was obtained while in 2 expts. the relaxation was much reduced when the incubation was carried out in the presence of 15-HPETE (Fig. 2).

DISCUSSION

The isolation of 6-keto-$PGF_{1\alpha}$ following incubation of arachidonic acid with homogenates of human umbilical artery shows that this tissue possesses the enzymes necessary for the formation of prostacyclin (PGI_2). In contrast to other arterial preparations investigated (Bunting et al 1976, Moncada et al. 1976) the human umbilical artery had a high capacity of formation of prostacyclin from exogenous arachidonic acid, i.e. the fatty acid cyclo-oxygenase activity was high. It is thus possible that synthesis of prostacyclin in vivo by the human umbilical artery is not dependent upon the availability of prostaglandin endoperoxides from e.g. platelets (cf. Bunting et al 1976, Tansik et al 1978) but is regulated by the phospholipase activity in the vessel.

That the relaxing agent formed from PGH on incubation with pig aorta microsome powder was identical with prostacyclin was supported by three findings, i.e. its instability (Bunting et al 1976, Moncada et al 1976), its inhibitory effect on platelet aggregation (Bunting et al 1976, Moncada et al 1976) and the inhibitory effect of 15-HPETE on its formation (Moncada et al 1976). We have earlier shown that the human umbilical artery produces TXB_2 (Tuvemo et al 1976) the stable end product of TXA_2.

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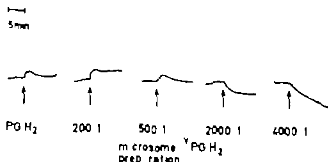


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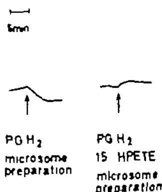


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Effect of substance P on CCK or VIP induced choleresis in anesthetized dogs

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MAGNUSSON (HOLM) I & THULIN L. Effect of substance P on CCK or VIP induced choleresis in anesthetized dogs. Acta Physiol Scand 1979 106 293–297 Received 22 Dec. 1978. ISSN 0001-6772. Department of Surgery Södersjukhuset, Department of Experimental Surgery Karolinska Institutet and Department of Surgery Serafimerlasarettet Stockholm Sweden

10 anesthetized dogs were provided with acute common bile duct fistulas and the gallbladder was excluded. Hepatic bile output and biliary content of sodium, potassium and amylase were studied. 6 caval infusions were administered of CCK 0.3 Ivy U kg^{-1} min^{-1} with a superimposed infusion of SP 20 ng kg^{-1} min^{-1}. 7 caval infusions were given of VIP 50 ng kg^{-1} min^{-1} with a superimposed infusion of SP 20 ng kg^{-1} min^{-1}. CCK increased bile output and biliary content of sodium, potassium and amylase by 78–110%. The corresponding increase induced by VIP was 55–85%. Biliary pH was not influenced. SP abolished the effect of both CCK and VIP. It is suggested that all peptides studied influenced canalicular bile secretion by changing the electrolyte excretion.

Key words. Substance P CCK, VIP choleresis

Substance P (SP) (Euler & Gaddum 1931 Chang & Leeman 1970) is an undecapeptide present in the central and peripheral nervous system as well as in smooth muscle organs. SP has been found to have neurotropic and motor actions and to increase secretion from salivary glands (Lembeck et al 1968) and pancreas (Starke et al 1968 Thulin & Holm 1977) in the dog. It also induces natriuresis in anesthetized dogs (Macfarlane Mills & Ward 1974). In contrast to these stimulatory effects, SP has been demonstrated to reduce hepatic bile output and biliary excretion of electrolytes in anesthetized dogs (Holm, Thulin & Hellgren 1978). This anticholeretic effect was suggested to be due to a reduction of the bile acid independent canalicular bile fraction and secondary to a reduction of the active sodium transport.

The aim of the present study was to further investigate the mechanism behind the anticholeretic effect of SP. An experimental model was designed where SP was matched against gut peptides known to stimulate bile flow. For this reason SP was infused together with cholecystokinin (CCK) and vasoactive intestinal peptide (VIP) which were administered at dosages previously known to increase hepatic bile output in anesthetized dogs (Thulin 1973 Thulin & Hellgren 1976)

MATERIAL AND METHODS

The peptides used in these studies were: SP (Chang & Leeman 1970) CCK (Jorpes & Mutt 1966) and VIP (Said & Mutt, 1972) SP was delivered frozen in sterile saline and CCK and VIP lyophilized. All fractions were dissolved in sterile saline immediately before use.

The experiments were performed in 10 medium sized mongrel dogs. General anesthesia was induced with pentothal sodium, 25 mg kg b w and maintained at steady state with repeated doses. The dogs were intubated endotracheally and ventilated by an Engström respirator with N_2O O_2. The abdomen was opened via a small subcostal incision and the gallbladder was excluded by ligation of the cystic duct. A common duct fistula was created by insertion of catheter into the common duct close to the liver hilus. Aortic mean blood pressure was recorded electromechanically via catheter in the femoral artery. Infusion catheters were inserted via femoral vein into the inferior caval vein.

After surgery and stabilization of hepatic bile output total of 13 expts. were performed. In 6 expts. CCK was given as caval infusions at the dosage of 0.3 Ivy U kg^{-1} min^{-1} during 4 consecutive periods of 10 min

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Effect of substance P on CCK or VIP induced choleresis in anesthetized dogs

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[illegible] output [illegible] (mmol/min)	Concentration (mmol/l)
[illegible]	2.5±0.32
[illegible]	2.2±0.32 N.S.
[illegible]	[illegible]1±0.27 [illegible] 0.05
[illegible]	[illegible]5±0.43 N.S. 6
[illegible]	2.7±0.41
[illegible]	[illegible]5±0.50 N.S.
[illegible]	[illegible]4±0.84 N.S.
[illegible]	2.0±0.44 N.S. 7

[illegible] *biliary concentration of sodium* varied by less than 4% during all expts and was thus unaffected by all peptides administered.

C. *Potassium*

Potassium was analyzed only in 3 CCK expts, but in all 7 VIP expts.

CCK increased the *total biliary potassium output* by a mean of 110% compared to the control level. When SP and CCK were administered together the potassium output was reduced to 18% below the control. After withdrawal of SP the output of potassium increased again to slightly below the previous CCK-induced level. The *biliary concentration of potassium* was unaffected by both CCK and the simultaneous administration of CCK and SP.

VIP increased the *total biliary potassium output* by 85% compared to the control. When SP and VIP were administered together the potassium output was reduced to the control level. After withdrawal of SP the output of potassium increased to a level which was 37% higher than that obtained during the previous VIP infusion ($P<0.01$). The *biliary concentration of potassium* was increased by VIP by 11%. When SP and VIP were administered together the potassium concentration decreased down to the control level. After termination of the SP infusion and during the consecutive VIP infusion the potassium concentration resumed its previous VIP-induced level.

D. *pH*

The pH in bile was analyzed in only 3 expts following CCK but in all 7 expts following VIP. It remained constant during all expts and was thus unaffected by all peptides administered.

E. *Amylase*

Both CCK and VIP increased the *total biliary amylase output* by a mean of 78 and 55% respectively. When SP was given simultaneously with CCK and VIP the amylase output decreased to or slightly below the control levels. After withdrawal of SP the amylase output resumed the levels obtained when CCK or VIP were given alone. The *biliary concentration of amylase* showed a slight decrease during simultaneous administration of CCK and SP but was otherwise unaffected by the peptides used.

F. *Blood pressure*

The mean aortic blood pressure before peptide infusions was 160 mmHg. Caval infusions of CCK and VIP both decreased the blood pressure by 15%. The combined infusion of CCK and SP promoted a further decrease in blood pressure by 35% while the corresponding additional decrease following the combined infusion of VIP and SP was 10%. When SP was withdrawn the blood pressure rised but remained slightly below the initial levels. Approximately 10 min after the end of the peptide infusions the blood pressure had resumed its control level.

DISCUSSION

The experimental procedure used in the present study was equal to that used previously for studying the anticholeretic effect of SP (Holm, Thulin & Hellgren 1978). The surgical preparation eliminated influences from the gallbladder and the extrahepatic bile ducts. The experiments were performed at brief intervals during a steady state of general anesthesia and they may be considered to have been performed during an intact entero-hepatic circulation. This is indicated by the fact that the bile flow was equal before and after the infusions and thus the

Table 1 *Effects of CCK VIP and SP on bile output biliary excretion of sodium potassium and amylase a billary pH*

Doses kg min CCK 0.3 Ivy U VIP 50 ng SP 20 ng. P values denote significances for changes from controls

			Sodium		Potassium		
		Bile output (g/10 min)	Total output (mmol 10 / 10 min)	Concentration (mmol/l)	Total output (mmol 10⁻ / 10 min)	Concentration (mmol/l)	pH
Control	$\bar{x}$±S E M	1 0±0 12	156±16.5	160±4 79	5 0±0 75	5 1±0 09	8 1±0
CCK	$\bar{x}$±S E M	1 9±0 17	316±32 6	16_±3 96	10 5±1 75	4.8±0 44	8 2±0
	P<	0 01	0 01	N S	N S	N.S	N.S
CCK and SP	$\bar{x}$±S E M	0 8±0 13	131± 1 8	164±3 68	4 1±0 74	4 8±0.32	8 2±0
	P<	N S	N S	N S	N.S	N.S	N S
CCK	$\bar{x}$±S E M	1 7±0 13	282±23 9	162±2._3	8 4±1 07	4.5±0.21	8.2±0 1
	P<	0 01	0 01	N S	N S	N.S	N S.
	n	6	6	6	3	3	3
Control	$\bar{x}$±S E M	1.2±0 12	197±22.3	170±4.24	6.1±0 74	5.2±0.28	8.1±0
VIP	$\bar{x}$±S E M	1 9±0 15	319±30.3	169±_.70	11 3±1 15	6.1±0.25	8.1±0
	P<	0 01	0 01	N S	0 01	0.01	N.S
VIP and SP	$\bar{x}$±S E.M	1 ±0 13	208± 4 8	176±2 76	7 0±0 86	6.0±0 37	8 1±0
	P<	N S	N S	N S	N S	N.S	N.S
VIP	$\bar{x}$±S E M	2 6±0 15	448± 4.5	175±2 98	15.5±0 85	6.1±0.38	8 1±0
	P<	0 001	0 001	N S	0 001	0 05	N S
	n	7	7	7	7	7	7

each. During the third period an infusion of SP at a dosage of 20 ng kg min was superimposed In 7 expts. VIP was given as caval infusions at the dosage of 50 ng kg min during 3 consecutive periods of 10 min each During the second period a caval infusion of SP at the dosage of 20 ng kg min was superimposed During control periods before and after the administration of peptides saline was infused in corresponding volumes

The parameters studied were hepatic bile output and biliary content of sodium potassium and amylase and biliary pH The bile output was measured by weighing the sodium and potassium content by flame photometry the amylase content by starch hydrolyzing capacity and the pH by a pH-meter

Statistical analysis of the data obtained was performed by the use of Student s t-test for correlated means A P value less than 0 01 was considered statistically significant and values less than 0 05 as a slight change

RESULTS

The results are given in Table 1

A *Hepatic bile output*

CCK at the dosage of 0 3 Ivy U kg min and VIP at the dosage of 50 ng kg⁻¹ min⁻¹ increased the bile output by a mean of 90 and 58 % respectively This increase in bile output was abolished by a superimposed infusion of SP 20 ng kg⁻¹ min⁻¹ resulting in a return of the bile output to the contro levels observed before administration of CCK o VIP After termination of the SP infusion, two dif ferent patterns were noted During the consecutiv CCK infusion the bile output returned to the previ ous CCK induced level During the consecutiv VIP infusion however the bile flow increased to a level which was significantly (P<0 01) higher tha the previous VIP induced level The mean differ ence in bile output between the VIP infusions be fore and after SP was 37 %

B *Sodium*

CCK and VIP increased the *total biliary sodium* output by a mean of 103 and 62 % respectively When SP was added to the CCK or VIP infusion the sodium output fell to the control levels Afte termination of the SP infusion and during the con secutive CCK administration the sodium outpu resumed its previous CCK induced level In con trast after termination of the SP infusion and du ing the consecutive administration of VIP th sodium output increased significantly (P<0 01) b 40 % above the previous VIP-induced level Th

plac

...al output ... $10^{-?}$ ...	Concentration (µkat/l)
[illegible]	2 5±0 32
[illegible]	2 2+0 32 N S.
[illegible]	2 1±0 77 0 05
[illegible]	5±0 45 N S 6
[illegible]	7±0 41
[illegible]	2 5±0.50 N S.
[illegible]	2.4±0 84 N S.
[illegible]	2 0+0 44 N S 7

...ean *biliary concentration of sodium* varied by less than 4% during all expts. and was thus unaffected by all peptides administered

C. Potassium

Potassium was analyzed only in 3 CCK expts. but in all 7 VIP expts

CCK increased the *total biliary potassium* output by a mean of 110% compared to the control level When SP and CCK were administered together the potassium output was reduced to 18% below the control. After withdrawal of SP the output of potassium increased again to slightly below the previous CCK-induced level The *biliary* ...*oncentration of potassium* was unaffected by both CCK and the simultaneous administration of CCK and SP

VIP increased the *total biliary pot ssium* output by 83% compared to the control. When SP and VIP were administered together the potassium output was reduced to the control level. After withdrawal of SP the output of potassium increased to a level which was 37% higher than that obtained during the previous VIP infusion ($P<0.01$) The *biliary concentration of potassium* was increased by VIP by 11%. When SP and VIP were administered together the potassium concentration decre sed down to the control level After termination of the SP infusion and during the consecutive VIP infusion the potassium concentration resumed its previous VIP induced level.

D *pH*

The pH in bile was analyzed in only 3 expts following CCK but in all 7 expts following VIP It remained constant during all expts and was thus unaffected by all peptides administered

E. *Amylas*

Both CCK and VIP increased the *total biliary am lase* output by a mean of 78 and 55% respectively When SP was given simultaneously with CCK and VIP the amylase output decreased to or slightly below the control levels. After withdrawal of SP the amylase output resumed the levels obtained when CCK or VIP were gi en alone The *biliary on entration of amyl* showed a slight decrease during simultaneous administration of CCK and SP but was otherwise unaffected by the peptides used

F *Blood pressure*

The mean aortic blood pressure before peptide infusions was 160 mmHg. Caval infusion of CCK and VIP both decreased the blood pressure by 15% The combined infusion of CCK and SP promoted a further decrease in blood pressure by 35% while the corresponding additional decrease following the combined infusion of VIP and SP was 10% When SP was withdrawn the blood pressure rised but remained slightly below the initial levels Approximately 10 min after the end of the peptide infusions the blood pressure had resumed its control levels

DISCUSSION

The experimental procedure used in the present study was equal to that used previously for studying the anticholeretic effect of SP (Holm Thulin & Hellgren 1978) The surgical preparation eliminated influences from the gallbladde and the extrahepatic bile ducts. The experiments were performed at brief intervals during a steady state of general anesthesia and they may be considered to have been performed during an intact entero-hepatic circulation This is indicated by the fact that the bile flow was equal before and after the infusions and thus the

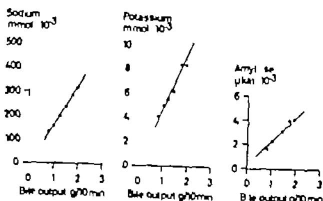


Fig 1 Relationship between bile output and biliary excretion of sodium potassium and amylase Each point represents the mean value of 6 analyses of sodium and amylase and 3 analyses of potassium during infusion of saline CCK or CCK+SP Sodium $r=0\ 998$ Potassium $r=0\ 967$ Amylase $r=0\ 946$

excretion of bile acids constituting a driving force for choleresis was not impoverished

The finding in the present work of an increase in bile output induced by CCK and VIP agrees with earlier findings in anesthetized dogs (Thulin 1973 Thulin & Hellgren 1976) Following CCK there was a latency of approximately 10 min for inducing a steady state of bile secretion Therefore the first 10 min period of CCK infusion was omitted and the second 10 min period was considered to represent the CCK effect VIP at the dosage used has been demonstrated to give a maximum choleretic response which remains at a steady level during 10 min after withdrawal of the peptide SP was infused at a dosage which has been shown to depress bile flow by approximately 50% (Holm Thulin & Hellgren 1978)

The essential action of SP was a complete inhibition of CCK or VIP-induced effects which resulted in a return of the output of hepatic bile and biliary content of sodium potassium and amylase to the control levels When SP was withdrawn but CCK or VIP still administered two different patterns of bile secretion were noticed Thus during the consecutive CCK infusion all parameters returned to the previous CCK-induced levels During the consecutive VIP infusion on the other hand the output of bile sodium and potassium increased above the previous VIP induced levels SP has been demonstrated to have a biphasic action on bile flow an anticholeretic effect during infusion and a choleretic effect after termination of infusion (Holm Thulin & Hellgren 1978) The fact that this biphasic action of SP was demonstrated only against VIP could be due to the weaker choleretic action of this peptide compared to CCK. This quantitatively different pattern of interaction between SP and CCK respectively VIP might also be suggested to depend on structural similarity and interaction on the receptor sites (Grossman 1973). However CCK and VIP are structurally mutually dissimilar and SP is structually dissimilar to both CCK and VIP (Mutt personal communication).

The mechanism behind the anticholeretic effect of SP was studied by matching it against the choleretic effects of CCK and VIP It seems reasonable that CCK and VIP together with other peptide hormones take part in the regulation of hepatic bile output although their mode of action is not very well studied previously In this context their possible influence on the various bile fractions should be considered The total hepatic bile secretion is believed to be the result of 3 different secretory mechanisms affecting different bile fractions (Erlinger et al 1970 Erlinger & Dhumeaux 1974). One is supposed to be bile acid dependent and of canalicular origin a second fraction seems to be sodium dependent and also of canalicular origin and a third fraction is believed to be regulated by secretin and of ductular-ductal origin It has been demonstrated that changes in biliary pH are correlated to the secretin-promoted ductular-ductal bile fraction (Wheeler & Ramos 1960) In the present experiments the pH was found not to be influenced by any of the peptides used This indicates that neither CCK nor VIP which is secretin like nor SP influenced the bile production at the ductular-ductal level

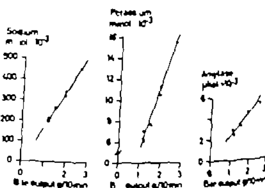


Fig 2 Relationship between bile output and biliary excretion of sodium potassium and amylase Each point represents the mean value of 7 analyses during infusion of saline VIP or VIP+SP Sodium $r=0.999$ Potassium $r=0\ 988$ Amylase $r=0\ 949$

SP has previously been suggested to reduce the sodium-dependent fraction of canalicular bile. In the present study it was demonstrated that there was a close relationship between bile output and biliary content of sodium and potassium as demonstrated by the regression lines for these factors both during the CCK- and VIP-induced choleresis and during the SP-induced anticholeresis (Figs. 1 and 2). The finding of a close relationship between bile output and biliary content of electrolytes indicates that all peptides influenced the bile acid independent bile fraction at the canalicular level by changing the excretion of electrolytes. An additional effect on the bile acid dependent bile fraction cannot be ruled out by the present expts. and will therefore be the subject of further study.

In the literature we have found no mechanism postulated for the output of amylase in bile. However there was also a close relationship between bile output and biliary content of amylase (Figs. 1 and 2). This might be due to an effect of the peptides on an amylase secretory mechanism, or that the amylase output is dependent on for instance the sodium excretion, or that the amylase output is regulated by osmosis.

SP was synthetized and kindly supplied by Dr Karl Folkers, Institute for Biomedical Research, Austin, Texas, USA. Pure natural CCK and VIP were kindly supplied by Prof Viktor Mutt, GIH Research Unit, Chemistry Department, Karolinska Institutet, Stockholm, Sweden.
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ffects of 1,3-diaminopropane on testosterone induced ypertrophy and polyamine synthesis in mouse kidney

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CAMERON R., HENNINGSSON S PERSSON L. & ROSENGREN E. Effect of 1,3-diaminopropane on testosterone induced hypertrophy and polyamine synthesis in mouse kidney Acta Physiol Scand 1979 106. 299–305 Received 7 Dec. 1978 ISSN 0001 6772. Department of Pathology and Department of Physiology University of Lund, Sweden.

The effects of prolonged treatment with 1,3-diaminopropane structural analogue of putrescine on polyamine metabolism and growth in kidney tissue, were studied in mice in which renal hypertrophy was induced by testosterone treatment. Injections of 1,3-diaminopropane resulted in an almost total suppression of the testosterone induced stimulation of ornithine decarboxylase activity and prevented the accumulation of putrescine and spermidine in the kidneys. Renal spermine concentration was even lowered. Administration of 1,3-diaminopropane effectively prevented the testosterone induced increase in renal weight and RNA. I mice receiving 1,3-diaminopropane proteinuria was observed and histological examination revealed renal damage. Due to the nephrotoxic action of 1,3-diaminopropane caution is essential in relating the prevention of renal hypertrophy and the inhibition of polyamine synthesis.

Key ords Polyamines, 1,3-diaminopropane, renal hypertrophy renal toxicity

e polyamines putrescine spermidine and sper ne are referred to in conjunction with rapid wth of cells and tissue An increased biosynthesis and elevated levels of polyamines are consist t observations in the early phases of growth, e g. regenerating rat liver in cell cultures stimulated growth, in developmental and malignant growth r ref see Jänne, Pösö & Raina 1978)

We have previously reported that administration testosterone propionate to castrated mice be des giving a distinct hypertrophy of the kidneys sulted in a dramatic change in the metabolism of lyamines (Henningsson, Persson & Rosengren 78) Within a few days of testosterone treatment e activity of ornithine decarboxylase [EC 4 1.1.17], considered to be the rate limiting step in biosynthesis of polyamines, attained values well ove 1000 times that of the controls. The increase (enzyme activity was accompanied by an accumulation of putrescine spermidine and spermine in the kidney

The physiological functions of the polyamines are still unknown although numerous biological effects of the amines have been reported (for ref see Raina & Jänne 1975) The use of inhibitors of polyamine synthesis could be helpful in elucidating the biological significance of these amines. Pösö & Jänne (1976*a*) took advantage of the fact that ornithine decarboxylase activity in vivo is regulated through a repression type mechanism not only by putrescine and spermidine but also by the more unphysiological 1,3-diaminopropane Repeated injections of 1,3-diaminopropane to partially hepatectomized rats prevented the early induction of ornithine decarboxylase as well as the accumulation of putrescine and spermidine in the liver remnant (Pösö & Jänne 1976*b*) Furthermore, the inhibition of polyamine synthesis by 1,3-diaminopropane was associated with a marked decrease in the synthesis of DNA whereas the synthesis of RNA and of total liver protein was unaffected

We have already shown that injections of 1,3-

diaminopropane to testosterone substituted mice significantly inhibited renal ornithine decarboxylase activity (Persson & Rosengren 1978) In addition to the effects on polyamine metabolism 1,3-diaminopropane has also been shown to possess a nephrotoxic action (Tabor & Rosenthal 1956) We therefore undertook a more detailed study of the effects of prolonged treatment with 1,3-diaminopropane on renal growth and polyamine metabolism in testosterone substituted mice at the same time examining the kidneys histologically

METHODS

Male mice of the NMRI strain were used They were fed a standard pellet diet and had water ad libitum Gonadectomy was performed at the age of 8 weeks Four weeks after gonadectomy the mice were given 3 daily subcutaneous injections of 200 μg testosterone propionate (British Drug Houses Ltd Poole England) suspended in 50 μl of arachis oil Controls were given arachis oil only 1,3-Diaminopropane was neutralized before use and administered intraperitoneally (150 μmol/100 g) every sixth hour from the first injection of testosterone onwards.

Preparation of samples for analysis

Mice were stunned and exsanguinated 77 h after the first injection The kidneys were rapidly removed and dissected free of the capsules For preparation of enzyme extracts kidneys were minced with scissors and then gently homogenized in a Dounce type homogenizer (25 strokes with the pestle) in 7 volumes of cold 0 1 M sodium phosphate buffer (pH 7 2) containing 10^{-} M EDTA 5×10^{-4} M dithiothreitol and 0 2% (w/v) glucose The homogenate was centrifuged at 20000 g for 20 min at 4°C The supernatant was used as the source of enzyme

Extracts for quantitative determination of polyamines were prepared by homogenizing the minced kidneys in 9 volumes of a solution of 4% sulfosalisylic acid and 0 04% EDTA The extract was boiled on a waterbath for 30 min whereupon the mixture was chilled and centrifuged The pH of the sample was adjusted to 2 0–2.5 with NaOH and the extract was then filtered through a filter with a pore size of 0.22 μm (Millipore Corp Bedford Mass 01730)

Assay for ornithine decarboxylase activity

Ornithine decarboxylase activity was determined by measuring the release of $^{14}CO_2$ from carboxyl-labelled ornithine An aliquot of the enzyme extract corresponding to 2.5 mg of tissue (80 mg for the control) was incubated i a reaction mixture containing 10^{-4} M DL 1 ^{14}C-ornithine (sp act. 0.5 mCi/mmol or 5 mCi/mmol for the controls) 10^{-4} M pyridoxal-5-phosphate and the same phosphate buffer as used for homogenization in a final volume of 1 0 ml. After 30 min of incubation (controls 60 min) at 37°C the reaction was terminated by adding 0 5 ml of 2 M perchloric acid The expelled $^{14}CO_2$ was trapped on a 10×25 mm piece of No 005 Munktell filter paper prepared with 100 μl of hydroxide of Hyamine 10-X Maximal absorption of $^{14}CO_2$ was achieved by continued shaking for an additional min The filter paper was then placed in a vial containing ml of a liquid scintillation mixture (Bray 1960) and t radioactivity was measured in a Packard Tri-Carb liq scintillation spectrometer All values obtained were co rrected against a reaction mixture without enzyme Enzyme activity is expressed in pmol CO_2 formed per r soluble protein and hour

Assay for S-adenosyl-L-methionine decarboxylase activity

Enzyme activity was determined by measuring the l eration of $^{14}CO_2$ from carboxyl-labelled S-adenosyl methionine in the presence of putrescine as described Pegg & Williams-Ashman (1969) The incubation mix consisted of supernatant corresponding to 100 mg tissue 2×10^{-4} M C^{14}-carboxyl-labelled S-adenosyl methionine (sp act 0.2 mCi/mmol) 2.5×10^{-3} M putre cine and the same phosphate buffer as used f homogenization in a final volume of 1.0 ml The mix was incubated for 1 h at 37°C The reaction was ter nated by adding 0 5 ml of 2 M perchloric acid 1 evolved ^{14}CO was trapped and counted as descri above for the assay of ornithine decarboxylase acti All values obtained were corrected against a reaction m ture without enzyme Enzyme activity is expresse pmol CO_2 formed per mg soluble protein and hour

Quantitative determination of polyamines

Chromatographic separation and quantitative estima of the amines in kidney extracts were carried out by l resolution liquid chromatography using the autom amino acid analyzer LKB-BIOCAL 3201 A mod (Persson & Rosengren 1979) method of Kremzner (l was used to achieve separation between putrescine 1,3-diaminopropane

The chromatographic peak of 1,3-diaminopropa kidney extracts of animals not injected with the amine small Consequently we have not been able to isola 1,3-diaminopropane fraction and compare the adequa the resolution with other established procedures fo termination of 1,3-diaminopropane Thus the de identification of endogenous 1,3-diaminopropane in ney extracts remains to be achieved

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The extraction of RNA and DNA was performed b Schmidt & Thannhauser (1945) procedure as modifi Schneider (1946) The amounts of RNA and DNA then estimated using the orcinol method (Mejbaum and the diphenylamine method (Burton 1956, Ric 1974) respectively These procedures for separatio determination of nucleic acids as used in this labo have been described (Henningsson et al 1978)

Protein from tissue and urine was measured b method of Lowry et al (1951)

Preparation of samples for histological examination

Kidneys were fixed whole in 10% neutral formalin. l sections from each kidney were processed in an technicon model 2A tissue processor and 2 3 μm se

Table 1. *Effects of prolonged treatment with 1,3-diaminopropane on the activities of renal ornithine and adenosyl-L-methionine decarboxylase*

Castrated mice received 3 daily injections of 200 μg testosterone propionate. 1,3-Diaminopropane (150 μmol/100 g b wt.) administered every 6 h to half of the group receiving testosterone. Mean and S.E. of mean are given n=6

Treatment	Ornithine decarboxylase activity (nmol/mg protein/h)	S-Adenosyl-L-methionine decarboxylase activity (pmol/mg protein/h)
Castrated controls	0.02±0.004	254.8±30.45
Testosterone	160.9±7.84	273.8 14.37
Testosterone+1,3-diaminopropane	3.5±2.72***	447.6_39.54*

P<0.01 *** P<0.001 (as compared with the group receiving testosterone only).

... cut on ... Leitz 1400 microtome. Sections were stained with hematoxylin and eosin and with periodic acid-Schiff reagent. Animals were coded and slides examined without previous knowledge of the experimental group. Sections were evaluated with respect to type and severity of glomerular, tubular and interstitial changes.

RESULTS

Effects of 1,3-diaminopropane on the activities of ornithine and S-adenosyl-L-methionine decarboxylases

Table 1 shows the activities of renal ornithine and S-adenosyl-L-methionine decarboxylases in testosterone-substituted mice (200 μg/day) after repeated injections of 1,3-diaminopropane (150 μmol/100 g) every 6 h. Testosterone treatment only resulted in a striking increase in ornithine decarboxylase activity in the kidneys of castrated mice. The enzyme activity rose from 0.02 nmol/mg protein/h to 161 nmol/mg protein/h. Administration of 1,3-diaminopropane prevented this testosterone induced stimulation of kidney *ornithine decarboxylase* activity; only about 2% of the enzyme activity persisted on treatment with 1,3-diaminopropane.

Testosterone administration to mice did not markedly affect the activity of S-adenosyl-L-methionine decarboxylase in the kidneys. On the other hand if 1,3-diaminopropane (150 μmol/100 g) in addition to testosterone treatment was injected every 6 h an increase in enzyme activity of more than 60% was observed (Table 1).

Effects of 1,3-diaminopropane on renal polyamine concentrations

The effects of prolonged treatment with 1,3-diaminopropane on renal polyamine concentrations are seen in Table 2. Testosterone administration for 3 days to castrated mice resulted in an increase in the concentrations of putrescine and spermidine whereas spermine concentration remained unchanged. Probably as a result of decreased ornithine decarboxylase activity in the kidneys of mice treated with both testosterone propionate and 1,3-diaminopropane the putrescine concentration was considerably lower than in the kidneys of mice treated with testosterone only. However even though 1,3-diaminopropane administration restrained the increase in renal putrescine concentration it did not

Table 2. *Effects of prolonged treatment with 1,3-diaminopropane on renal polyamine concentrations*

Castrated mice were injected with testosterone propionate and 1,3-diaminopropane as described in Table 1. The concentration of polyamines are given in nmol/g. Mean and S.E. of mean are given, n=6

Treatment	1,3-Diaminopropane	Putrescine	Spermidine	Spermine
Castrated controls	8±1.0*	16±2.4**	386±16.7***	778±61.6
Testosterone	17±3.0	350±35.2	513±21.4	743±56.8
Testosterone+1,3-diaminopropane	1 319±212.3	82±35.6 **	383±37.5	423±27.7***

* P<0.05 *** P<0.001 (as compared with the group receiving testosterone only)

diaminopropane to testosterone substituted mice significantly inhibited renal ornithine decarboxylase activity (Persson & Rosengren 1978) In addition to the effects on polyamine metabolism 1,3-diaminopropane has also been shown to possess a nephrotoxic action (Tabor & Rosenthal 1956) We therefore undertook a more detailed study of the effects of prolonged treatment with 1,3-diaminopropane on renal growth and polyamine metabolism in testosterone substituted mice at the same time examining the kidneys histologically

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Preparation of samples for histological examination

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Table 3. *Effects of prolonged treatment with 1,3-diaminopropane on renal growth*

Castrated mice were injected with testosterone propionate and 1,3-diaminopropane as described in Table 1. Mean and SE of mean are given, n=6 except for kidney weight which are the mean of 22 observations

Treatment	Kidney weight (mg)	RNA conc (mg/g)	RNA total (mg)	DNA conc (mg/g)	DNA total (mg)
Castrated controls	311±6.3	6.[illegible]±0.09*	1.9±0.05**	7.8±0.14**	2.4±0.07
Testosterone	382±6.5	7.7±0.17	3.0±0.07	5.8±0.14	2.3±0.04
Testosterone 1,3-diaminopropane	306±7.9***	4.7±0.32*	1.4±0.10*	7.1±0.[illegible]1	2.2±0.09

P<0.001 (as compared with the group receiving testosterone only).

[illegible] of 1,3-diaminopropane resulted in a considerable accumulation of this amine in the kidneys.

Effect of 1,3-diaminopropane on testosterone induced renal hypertrophy

Testosterone administration to castrated mice gave rise to a marked growth of the kidneys. The kidney weight was enhanced from 311 mg to 382 mg (Table 3). Repeated injections of 1,3-diaminopropane every 6 h completely prevented this increase in kidney weight; the kidneys of mice treated with both testosterone propionate and 1,3-diaminopropane did not differ in weight from the mice treated with vehicle only (Table 3).

As shown in Table 3 the total amount of DNA in the kidneys was unaffected by testosterone administration whereas the concentration of DNA was decreased as a result of the growth of the kidneys. The concentration as well as the total amount of RNA, on the other hand, were both increased in the mouse kidney after testosterone treatment. These changes in the levels of nucleic acids confirm earlier results (Kochakian & Harrison 1962, Henningsson et al. 1978) that the kidneys under androgen stimulation hypertrophy without an appreciable change in DNA content.

Administration of 1,3-diaminopropane effectively prevented the testosterone induced increase in renal RNA content, in fact the RNA levels in the kidneys of mice after administration of both testosterone and the inhibitor were even lower than in castrated controls (Table 3).

In contrast to the effect on renal RNA content, treatment with 1,3-diaminopropane did not obviously affect the amounts of DNA (Table 3).

Protein concentration was unaffected by 1,3-diaminopropane administration (figures not included).

Nephrotoxic effect of 1,3-diaminopropan

The histological findings fell into 3 fairly well defined groups which paralleled the experimental grouping (Fig. 1). Mice injected with arachis oil alone showed normal renal histology. Those injected with testosterone showed a general hypertrophy and a mild but definite cloudy swelling of tubular epithelium. Mice receiving 1,3-diaminopropane showed severe changes in the proximal tubules primarily in the outer stripe. Here the epithelium was necrotic or had disappeared and tubules were dilated. In the interstitium around the damaged tubules oedema and a moderate to pronounced inflammatory infiltrate composed primarily of polymorphonuclear leucocytes with occasional lymphocytes and plasma cells was seen. In some tubules a

Table 4. *Effect of prolonged treatment with 1,3-diaminopropane on total urinary protein excretion (mg protein/24 h)*

Castrated mice were injected with testosterone propionate and 1,3-diaminopropane as described in Table 1. Mean and SE of mean are given, n=4

Treatment	Day 1	Day 2	Day 3
Castrated controls	7.[illegible]±0.94	7.7±0.57	8.7±0.49
Testosterone	7.3±0.86	6.5±1.13	7.2±1.47
Testosterone 1,3-diaminopropane	9.6±1.43	17.4±2.60*	23.7±3.48**

** P<0.01 (as compared with the group receiving testosterone only).

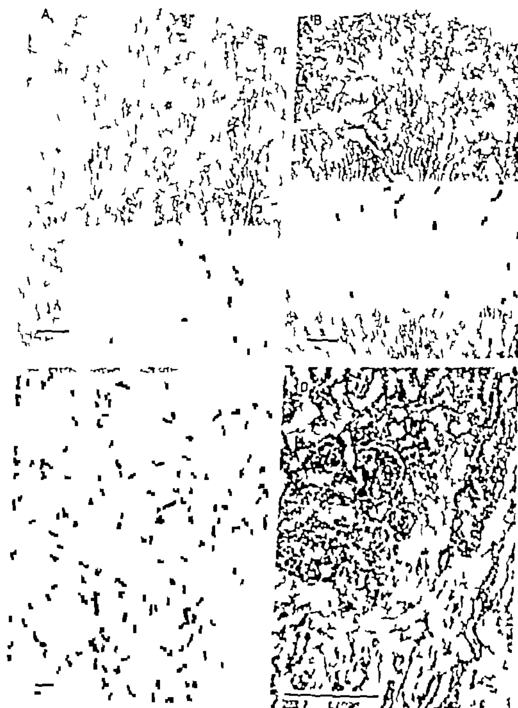


Fig 1 Cortex and outer medulla from. (A) Mouse injected with arachis oil only normal renal histology (B) M[illegible] treated with testosterone minimal changes in proximal tubules. (C) Mouse treated with testosterone and [illegible] diaminopropane degeneration of tubule epithelium in the outer medullary stripe (D) Higher magnification of (C) sh[illegible] advanced degeneration and desquamation of tubule epithelium *Sections H/E Markers = 200 μm*

completely prevent the enhancement Renal putrescine concentration was increased 5 times after administration of both testosterone propionate and 1,3-diaminopropane as compared with 22 times after testosterone administration only

Prolonged treatment with 1,3-diaminopropane totally abolished the testosterone stimulated increase in kidney spermidine concentration Renal sp[illegible] ine concentration was markedly decreased after [illegible] longed treatment with 1,3-diaminopropane [illegible] spermine concentration decreased more than [illegible] when 1,3-diaminopropane was administered to testosterone substituted mice (Table 2)

It is further shown in Table 2 that repeated in[illegible]

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regeneration of epithelium could be seen Tubules in the inner stripe were well preserved but often contained eosinophilic fluid Glomerular changes in this group were discrete consisting of a slight increase in the number of mesangial cells and the appearance of occasional inflammatory cells

A marked and progressive increase in urinary protein loss in mice given 1,3-diaminopropane (Table 4) confirmed the histological findings of a possible tubule damage

DISCUSSION

In order to elucidate the biological functions of polyamines the use of specific inhibitors of polyamine synthesis would be of great value Several compounds have been used with variable success to block the accumulation of polyamines that normally occurs in rapidly growing tissues and cells L Ethionine α hydrazinoornithine methylglyoxal bis (guanyl hydrazone) α methylornithine and DL-α hydrazino-δ-aminovaleric acid are among the inhibitors employed (for ref see Jänne et al 1978) Recently Pösö & Jänne (1976*a*) approached the problem of inhibiting polyamine synthesis in a new way The key enzyme in polyamine synthesis ornithine decarboxylase is known to be regulated through a "repression type" mechanism by putrescine and spermidine (Kay & Lindsay 1973 Jänne & Hölttä 1974) Also a structural analogue of putrescine 1,3-diaminopropane has been shown to possess this regulatory property However as distinguished from putrescine 1 3-diaminopropane is not converted to higher polyamines and could therefore as shown by Pösö & Jänne (1976*a*) function as an in vivo inhibitor of polyamine synthesis Administration of 1,3-diaminopropane to rats after partial hepatectomy caused a distinct inhibition of ornithine decarboxylase activity and prevented the accumulation of putrescine and spermidine in the regenerating liver Further injections of 1,3-diaminopropane resulted in an almost total suppression of the stimulation of DNA synthesis in the regenerating rat liver (Pösö & Jänne 1976*b*)

In agreement with earlier observations (Henningsson et al 1978) renal ornithine decarboxylase activity was strikingly increased in castrated mice after testosterone treatment Repeated administration of 1 3-diaminopropane prevented this stimulation of ornithine decarboxylase activity As the inhibition was not complete an increase in renal putrescine concentration was noted after administration of both 1,3-diaminopropane and testosterone Interestingly 1,3-diaminopropane treatment also resulted in a 60% enhancement of the activity of S-adenosyl-L methionine decarboxylase Although putrescine concentration and S-adenosyl-L-methionine decarboxylase activity were increased 1,3-diaminopropane injections restrained the testosterone induced accumulation of spermidine and even gave rise to a significant decrease in the concentration of spermine In this connexion it should be mentioned that urinary spermidine excretion was enhanced after treatment with 1,3-diaminopropane (to be published) A more detailed study of the effects of the inhibitor on the synthesis of spermidine and spermine might be justified As to the renal growth following testosterone substitution 1,3-diaminopropane treatment totally abolished the increase in kidney weight and decreased renal RNA content whereas that of DNA was unaffected

The histological changes are essentially the same as those detailed by Fischer & Rosenthal (1954) following injection with spermine They suggested that the toxicity might be due to a metabolic degradation product and Tabor & Rosenthal (1956) subsequently reported renal damage resulting in proteinuria with a variety of aliphatic amines, among them 1 3-diaminopropane No pathological changes other than an elevation in the leucocyte count were reported by these workers There is no obvious relationship between the toxic effects on proximal tubules and the remarkable inhibition of testosterone induced polyamine synthesis exerted by 1 3-diaminopropane The inhibitory effects exerted by 1 3-diaminopropane on polyamine metabolism in other organs such as liver ovary and thyroid (Pösö & Jänne 1976*b* Guha & Jänne 1977 Friedman et al 1977) speak against such a relationship Some caution is advisable in relating the prevention of renal hypertrophy by 1 3-diaminopropane and its effect on polyamine metabolism Further studies with various other renal toxic agents in mice substituted with testosterone may shed light on this problem None the less the use of repression type inhibitors may be of value in studies of the biological significance of polyamines

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Human forearm and kidney conversion of arachidonic acid to prostaglandins

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NOWAK, J & WENNMALM Å. Human forearm and kidney conversion of arachidonic acid to prostaglandins. Acta Physiol Scand 1979 106: 307–312. Received 15 Jan. 1979 ISSN 0001-6772. Department of Clinical Physiology Karolinska Institute, Huddinge Hospital, Sweden

The capacity of the human forearm and kidney to synthetize different prostaglandins (PGs) was studied together with the quantitative relationship between the various PGs formed in these organs. ^{14}C-labelled arachidonic acid (^{14}C AA) was infused in healthy male volunteers at constant rate into the brachial or the renal artery with simultaneous sampling of regional venous blood. The venous plasma content of ^{14}C-PGs was extracted, separated with thin-layer chromatography (TLC) and quantified using fractionated liquid scintillation spectrometry. Most of the ^{14}C AA infused was metabolized and radiopeaks parallel to unlabelled standards of PGD_2, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and 13 14-dihydro-15-keto-PGE_2 (Me) were obtained. The chromatograms of both the forearm and the kidney plasma contained all the peaks described but the relative distribution of the ^{14}C PGs differed between the two tissues. In the cubital venous plasma, the main PG (apart from Me) was 6-keto-$PGF_{1\alpha}$ indicating considerable synthesis of PGI_2 in the forearm. In the renal venous plasma, on the other hand, PGD_2 accounted for the largest part of the authentic ^{14}C-PGs found. Besides the tissue differences large inter-individual variations were observed. The results demonstrate the existence of both considerable tissue specificity and an appreciable inter-individual variation in the local conversion of AA to PGs in man.

Key words: Arachidonic acid, kidney prostaglandin prostacyclin, renal vasculature, skeletal muscle vascular tissue

ndogenous prostaglandins (PG) have been pro-
sed as mediators of several physiological and
thophysiological processes in man (Karim 1976)
d their appearance in the peripheral venous blood
s been studied intensively in recent years. Due to
e rapid release of PG from blood cells and from
jured tissue the analysis of venous plasma PG-
ncentrations may yield falsely high values and
ay therefore (unless extraordinary precautions are
ken during the sampling process) be a poor
ethod for estimating the endogenous PG-synth-
sis. A better parameter than the parent prostaglan-
ins in monitoring the endogenous formation of PGs
the stable 13 14-dihydro-15-keto derivative of the
spective compound (Samuelsson & Gréen 1974)
owever since these derivatives are probably
rmed in the blood from the respective parent PGs
eir plasma concentrations give no information ab-
ut the specific origin of the PGs formed. Studies on the local arachidonic acid (AA) metabolism in various parts of the circulatory system are therefore of interest, since the results may reveal a specific tissue differentiation of the endogenous PG synthesis, which in turn may contribute to an understanding of a possibly physiological role of the endogenous PGs. In the present investigation we have therefore studied the regional conversion of arachidonic acid to PGs in the human forearm and kidney.

MATERIAL AND METHODS

The study was performed with the permission of the Ethical Committee at Karolinska Institute.

19 healthy volunteers, all men aged 19–43 years, were studied. They were fully informed about the aim, experimental procedure and possible risks of the investigation before giving their voluntary consent to participate. All experiments were performed in the morning in supine body position after an overnight fast.

Table 1 *Thin layer chromatographic separation of PGs*

Compound	R_f values System C	System BDA	System A I	System A IX
6-keto-PGF	0.34–0.44		0.37–0.46	0.13
PGF	0.27	0.21	0.37–0.46	0.17
PGE_2	0.34–0.44	0.35	0.56	0.25
PGD_2	0.34–0.44	0.47	0.66–0.80	0.36
PGA_2, PGB_2, 13, 14, 2H, 15-keto-PGE_2	0.52	0.55–0.72	0.66–0.80	0.43–0.57
AA	0.63	0.83	0.89	0.78

14-dihydro-15-keto-PGE_2 run almost in parallel. The zone corresponding to these compounds was therefore considered as the metabolite zone (Me)

Forearm and kidney conversion of ¹⁴C-AA

Infusion of ^{14}C AA through the renal and forearm vasculature resulted in the appearance of ^{14}C-labelled AA metabolites in the venous blood from both regions. Thin layer chromatography of the purified lipid extracts of the venous plasma revealed that the ^{14}C activity chromatographed in parallel to authentic prostaglandin markers. Further evidence that the radioactive products in these zones really were ^{14}C PGs was obtained by comparing their R_f values in different solvent systems with the authentic compounds The changes in chromatographic mobility of the radioactive products were similar to those of authentic markers.

The relative amount of the activity which appeared during the ^{14}C AA infusion identified as ^{14}C PG was almost the same in renal (41±5% $n=7$) as in forearm venous plasma (38±3% $n=8$) The activity chromatographed usually in 5 peaks corresponding to PGA_2/PGB_2/13 14-dihydro-15-keto-PGE_2 (Me) PGD_2 PGE_2 $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ However the relative distribution of ^{14}C PGs in venous plasma differed between the two vascular regions investigated In the forearm ven-

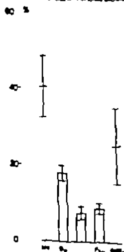


Fig 1 ^{14}C PG in forearm venous plasma during infusion of ^{14}C AA into the brachial artery in healthy males. The bars indicate mean ± S E. of the area of radiopeaks obtained in 4 expts (TLC, system AIX) and are expressed as a percentage of the total area of the ^{14}C PG peaks in the respective expts.

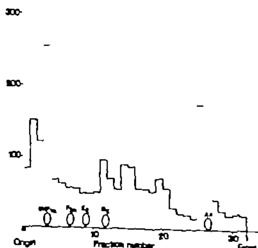


Fig 2 Typical radiochromatogram (TLC, system AIX) of the lipid extract from forearm venous plasma collected during infusion of ^{14}C-AA through the forearm vasculature in healthy volunteer Note the prominent 6-keto-$PGF_{1\alpha}$ peak, indicating that ^{14}C-PGI_2 was the main PG formed.

a) Catheterization and isotope infusion In 10 subjects aged 22–33 years a teflon catheter (outer diameter 1 mm) was inserted into a brachial artery Another similar catheter was inserted 50–60 mm in the retrograde direction from the ipsilateral antecubital vein so that the tip of the catheter was positioned deep in the forearm muscle This catheter position yields blood that is representative of the forearm venous effluent (Wahren 1966) After catheter insertion dynamic forearm work (handgrip) was performed for 15 min on a hand ergometer (Piab Sweden) The work load was 1 W and the contraction frequency 1 Hz. During the last 10 min of the forearm work 5 μCi ^{14}C arachidonic acid (^{14}C AA) (New England Nuclear sp act 40–60 mCi/mmol) freshly prepared as the sodium salt in saline was infused intra-arterially at a constant rate of 0 5 μCi/min Simultaneously forearm venous blood was sampled into 10 ml syringes at a constant sampling rate of 15–20 ml/min

In 9 subjects aged 19–43 years two teflon catheters (outer diameter 1 66 mm) were inserted into a femoral artery and a femoral vein respectively The arterial catheter was advanced under fluoroscopic control to one of the renal arteries and the venous catheter was passed to the ipsilateral renal vein After the position of the arterial catheter had been verified by the injection of a small amount of radiopaque medium (Isopaque Cerebral) the subjects rested comfortably and 10 μCi of ^{14}C-arachidonate was infused during 10 min into the renal artery at a constant rate of 1 0 μCi/min During the entire infusion renal venous blood was continuously sampled as described above to a final amount of 200 ml

All catheters were inserted percutaneously under local anesthesia. The patency of the catheters when not used for infusion of isotope or sampling of blood was maintained by intermittent flushing with saline

b) Extraction and separation of plasma ^{14}C PGs All subjects were pretreated with 3 g aspirin 3 days before the investigation to avoid release of prostaglandins from the platelets during sampling of blood Such treatment inhibits PG-synthesis by platelets for 1–4 days after administration of the drug (Kocsis et al 1973) without affecting the general PG-synthesis in the tissues (Hamberg 1972)

The venous blood was collected in chilled vacutainers containing ethylene-diamine-tetraacetic acid (EDTA) It was kept on ice until the plasma was separated (within 30 min after sampling) by 15 min centrifugation at 2 000×g at 4°C. After separation the plasma was pooled and immediately subjected to further analyses

After dilution of the plasma with volumes of water (to avoid foaming during the lipid extraction) neutral lipids were removed by shaking with an equal volume of petrol (40°–60°) Extraction of prostaglandins was performed twice at pH 3.5 with equal volumes of ethylacetate (Fisher E 145) After evaporation of the organic phase the residue was reconstituted in 5 ml of toluene ethylacetate (9 1 v/v) Purification of the samples was performed using silicic acid chromatography Silicic acid (Mallincrodt, 100 mesh) was activated at 150°C Microcolumns (5×45 mm) were prepared with toluene ethylacetate (9 1) After application of the sample the columns were washed with 15 ml of toluene ethylacetate (9 1) Elution was performed with 20 ml of methanol The methanol was evaporated and the residue applied to TLC plates (0.25 mm DC Fertigplatten Kieselgel F254 Merck) Occasionally purified lipid extracts of forearm or renal venous plasma from more than one patient were pooled before being subjected to TLC. The plates were run 18 cm against unlabelled standards of 6-keto-PGF$_{1\alpha}$, PGF$_{2\alpha}$ PGE$_2$, PGD$_2$ PGB$_2$, PGA$_2$, 13 14-dihydro-15-keto-PGE$_2$ (Me) and Na-arachidonate. The following solvents were used 1) ethylacetate acetic acid 2,2,4-trimethylpentane water (90 20 50 100, v/v organic phase AIX Hamberg and Samuelsson 1966); 2) benzene dioxane acetic acid (20 20 1 v/v A I Hamberg and Samuelsson 1966) 3) benzene dioxane acetic acid (10 10 1 v/v BDA, Isakson et al. 1977), chloroform methanol acetic acid water (90 8 1 0.8, v/v C Isakson et al. 1977).

After development of the plates the sample lanes were divided into 5 mm horizontal zones which were scraped off and eluted with ethanol After addition of Instagel, the activity of the ethanol eluates was counted in a liquid scintillation spectrometer (Intertechnique SL 4000) The unlabelled PG standards were developed using phosphomolybdic acid

c) Calculations The total ^{14}C-activity in the thin-layer chromatograms was estimated as the total area of all the fractions after subtraction of background activity The area of the various, clearly defined radiopeaks that corresponded to authentic PGs was calculated and expressed in percent of the total area of ^{14}C PGs in the respective chromatograms The entire ^{14}C PG activity in the chromatograms was also calculated in relation to the total ^{14}C-activity All values are given as mean±S.E. unless otherwise stated.

RESULTS

Extraction and purification

The efficiency of the lipid extraction procedure was checked by determining recovery of ^{3}H labelled PGF$_{2\alpha}$ PGE$_2$ PGA$_2$ and PGB$_2$ added to external standards The recovery ranged between 54±4% (n=3) for ^{3}H PGA$_2$ and 73±2% (n=3) for ^{3}H-PGE$_2$ and ^{3}H PGF$_{2\alpha}$ The recovery of external PG standards in the methanol eluate of the silicic acid columns was almost quantitative

Thin layer chromatographic separation

Thin layer chromatographic separation of PGs was studied in several solvent systems (Table I). In some of the systems used two or more compounds had almost identical R_f values. Consequently calculation of the respective ^{14}C PG areas was not possible in these cases and only the total ^{14}C-PG-activity of the chromatograms could be estimated Complete separation of PGs of the D E F and F$_1$ series was only obtained with solvent system AIX. However even in this system PGA$_2$ PGB$_2$ and 13,

and the plasma turnover rate of AA is about 1 6 µmol/min (Hagenfeldt & Wennmalm 1975) the current ^{14}C AA infusion should not have altered the basal AA turnover in the tissues studied. This is important, since an elevation of the plasma concentration of PG precursor might well induce such changes in the tissue conversion of ^{14}C AA that the resulting ^{14}C PG production does not reflect the normal basal synthesis in the organ.

Apart from this, the validity of the results as standards of regional PG formation rests on the assumption that recirculating ^{14}C PG of the D E F and I series—formed from ^{14}C AA in other organs or tissues—did not influence the present chromatograms to any considerable degree. It has been established that PGs are rapidly metabolized in the circulatory system by dehydration of the 15-hydroxy group and reduction of the Δ^{13} double bond (Hamberg & Samuelsson 1971 Granström 1972). This degradation is probably common to PGD, PGE$_2$, PGF$_{2\alpha}$ and PGI and results in formation of the respective 13 14-dihydro-15-keto-derivatives. Since these derivatives differ in chromatographic mobility from their parent compounds, it does not appear likely that tissues beside those specifically studied interfered—via recirculating ^{14}C-PGs—with the present chromatograms. On the other hand, the 13 14-dihydro-15-keto-derivatives can be expected to occur in considerably higher circulating concentrations due to the rapid metabolism of the parent compounds (Samuelsson Green 1974). It is therefore conceivable that the 2-like zone in present chromatograms reflects to if not mainly the metabolites of ^{14}C PG generated in other organs or tissues.

When estimating the relative amounts of ^{14}C PGs formed from the infused ^{14}C AA the efficiency of the lipid extraction and purification procedures must be considered. The recovery of the respective PGs differed in this respect, which may raise difficulties in defining precisely the relative amounts of the ^{14}C-PGs formed. However these differences were not large compared to the inter-individual variations and probably did not influence the characteristic chromatographic PG-profiles to any significant extent. Furthermore this objection is irrelevant when comparing the relative distribution of PG biosynthetic pathways in the vascular regions studied.

The present chromatograms demonstrate that both vascular regions studied possess the capacity to generate PGs of the D E, F and I series from exogenously administered arachidonate. These results are mainly in accordance with data from previous studies. However the appearance of 6-keto-PGF$_{1\alpha}$ indicating formation of PGI$_2$, is remarkable since all subjects were pre-treated with aspirin at a dose that should be sufficient to block the conversion of AA to PG endoperoxides in the platelets. It has been suggested that vascular endothelium is unable to carry out conversion of AA to PG endoperoxides and therefore is obliged to "borrow" preformed endoperoxides from platelets adhering to the vascular wall (Bunting et al. 1976). The current data however indicate that formation of PGI in the vascular bed is not completely dependent upon an intact function of the cyclo-oxygenase in the platelets. This is further supported by a recent demonstration that human endothelial cells synthetize PGI directly from exogenous arachidonate (Weksler et al. 1977).

It has been reported that inhibition of PG synthesis by indomethacin decreases functional hyperemia in the human forearm (Kilbom & Wennmalm 1976). Recently increased concentrations of radioimmunoactive PGE material were demonstrated in the venous blood from working skeletal muscle in man (Nowak & Wennmalm 1978 b). On the basis of these and other observations endogenously formed and released vasodilating PGs have been proposed to modulate local vascular resistance (Hedqvist 1972 Staszewska-Barczak & Vane 1975 Nowak & Wennmalm 1978 a). The current data are compatible with such a role for endogenous PGs and focus interest on PGI$_2$ as the quantitatively most important PG in the forearm vascular bed.

In the kidney the major prostaglandins have been reported to be PGE$_2$ PGF$_{2\alpha}$ and recently also PGD$_2$ (Lee et al 1967 Hamberg 1969 Blackwell et al. 1975 Friesinger et al. 1978) all of which are represented in the current chromatograms. Since PGI$_2$ has been shown to be synthetized by human renal cortical microsomes in vitro (Whorton et al. 1977) it seems likely that this PG is also synthetized in intact kidney. The occurrence of a radiopeak corresponding to 6-keto-PGF$_{1\alpha}$ in the present chromatograms may provide further evidence for PGI$_2$ formation in the renal tissue although it is not yet known to what extent the renal vasculature accounts for this synthesis.

In summary the present data show that human

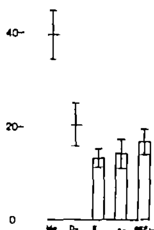


Fig 3 14C PG in renal venous plasma during infusion of 14C AA into a renal artery in healthy males The bars indicate mean ± S E of the area of radiopeaks obtained in 5 expts (TLC, system AIX) and are expressed as a percentage of the total area of the 14C PG peaks in the respective expts.

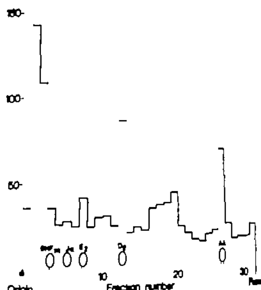


Fig 4 Typical radiochromatogram (TLC system A of the lipid extract from renal venous plasma collec during infusion of 14C AA through the renal vasculatur a healthy volunteer Note the major peak running par to PGD_2.

ous plasma (Fig 1) the major radiopeak (41±8%) was usually found in parallel to Me Large peaks were also found in parallel to 6-keto-$PGF_{1\alpha}$ (25±10%) and PGD_2 (18±2%) Two small radiopeaks displayed the same chromatographic behaviour as the $PGF_{2\alpha}$ (9±1%) and PGE_2 (8±2%) standards The ratio of $PGD_2/PGE_2/PGF_{2\alpha}$/ 6-keto-$PGF_{1\alpha}$ was 2 39 1 0 1 17 3 36 The most constant finding was the peak of radioactivity in parallel to PGD_2 (coefficient of variation 23%) whereas other 14C PGs showed larger quantitative inter individual variations The most pronounced interindividual differences occurred in the radioactive zone corresponding to 6-keto-$PGF_{1\alpha}$ (coefficient of variation 79%) Thus in one of the chromatograms (Fig. 2) 6-keto-$PGF_{1\alpha}$ was found to constitute 50% of the 14C PG activity compared with only 9% in the chromatogram from another experiment

Also in the renal venous plasma (Fig 3) the Me peak usually displayed the largest amplitude (40±5%) Major peaks were also found in parallel to PGD_2 (20±5%) and 6-keto-$PGF_{1\alpha}$ (17±3%) $PGF_{2\alpha}$ and PGE_2 appeared to be released in somewhat smaller and almost equal amounts (14±3% and 13±2% respectively) The ratio of PGD_2/ $PGE_2/PGF_{2\alpha}$/6-keto-$PGF_{1\alpha}$ was 1 54 1 0 1 07 1 Large inter-individual variations occurred in relative distribution of 14C PGs Thus, PGD_2 wh apart from Me was the main PG formed constit 32% of the 14C PG activity in one chromatogr (Fig. 4) compared with only 8% in another (co cient of variation 50%)

DISCUSSION

The aim of the present investigation was to find whether regional differences in the conversion 14C AA to 14C PGs occur in the vascular syst Such differences would imply that locally for PGs may play different roles in different parts of vascular bed As suitable regions we chose kidney and the forearm vasculature not only bec these regions are relatively simple to study but since they represent vascular beds that recei considerable part of the cardiac output

In the current experiments 14C labelled AA infused intraarterially The concentration of AA fused was low and the infusion rate did not exce and 17 nmol/min in the brachial and renal ar respectively Since the arterial plasma concen tion of AA in healthy young men is about 10 μm

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Ribonuclease activities in developing experimental granulation tissue with reference to polysomes

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The Mg^{2+}-precipitable polysomes in experimental granulation tissue increase up to three weeks and decrease slowly thereafter Polysomes from young granulation tissue are on a weight basis, more active in cell-free protein synthesis than polysomes from old granulation tissue RNase activity is highest in polysomes from 1-week tissue The polysomes contain both acid and alkaline RNase but the alkaline RNase is in latent form, manifested by the addition of p-chloromercuribenzoate (pCMB). The high RNase activity of granulation tissue renders the analysis of polysomal patterns and functions of the polysomes difficult. In the post-polysomal supernatant of granulation tissue the RNase activity is highest in 2–4 week old tissue it is maximal at pH 7–8 and only slightly influenced by pCMB

Key words Development, granuloma, polysomes, ribonuclease

In developing granulation tissue cell proliferation with the synthesis of nucleic acids, hyaluronate and cell proteins is followed by the synthesis of collagen and chondroitin sulphate and finally involution (Lampiaho & Kulonen 1967 Kulonen 1970. In this work we have investigated the RNase activities of the granulation tissue and related them to the RNA content and the capacity of the tissue for protein synthesis.

The cellular protein synthesis and hence the cell proliferation is indirectly controlled by the level of cellular RNA, altered by changes in either synthesis or degradation. The RNase inhibitor from liver supernatant stabilizes polysomes during the isolation (Bont et al. 1965 Gribnau et al. 1969) The cellular RNase/RNase-inhibitor system is thus involved in the regulation of protein synthesis (Kraft & Shortman 1970*a* Lau et al. 1975 Liu & Matrisian 1977 Blackburn et al 1977)

MATERIAL AND METHODS

Experimental granulation tissue Granulation tissue was induced by subcutaneous implantation of viscose cellulose-sponge into 3-month-old female albino rats (Viljanto & Kulonen 1962) T or three weeks after the implantation the granulomas were removed. They were processed fresh and the preparations stored frozen.

Isolation of the polysome for protein synthesis and RNase assay Polysomes were isolated with Mg^{2+}-precipitation (Palmiter 1974 Aho & Kulonen 1977). 1 g of tissue was homogenized into 5 ml of 25 mM tris-buffer pH 7.5 containing 25 mM NaCl, 5 mM $MgCl_2$, 1 mg/ml heparin and 2% Triton X-100. The homogenate was centrifuged at 27000 *g* for 10 min, after which the supernatant was diluted with 4/5 vol. of the homogenization buffer plus 1/5 vol. of 1 M $MgCl_2$, and incubated at 0°C for 1 h. The precipitated polysomes were collected by similar centrifugation through 10-ml cushion of sucrose (10% sucrose, 25 mM tris-buffer pH 7.5 25 mM NaCl, 5 mM $MgCl_2$). After the centrifugation the supernatant was removed by aspiration the upper part of the tube was washed with water which was removed by aspiration, and the sucrose layer was decanted off. The walls were wiped dry and the pellet was suspended in water to the concentration of 50 A_{260}-units/ml and stored in liquid nitrogen.

Preparation of the polysomes for the sedimentation profiles About 15 g of 3-week-old granulation tissue was homogenized with the Ultra-Turrax homogenizer (Janke & Kunkel, Staufen i Breisgau, Germany) into 10 ml of medium which contained one part 50 mM tris-buffer pH 7.4 10 mM magnesium acetate 1 M KCl and 120 mM sucrose plus one part of the liver supernatant prepared according to Gagnon & de Lamirande (1973) for the inhibition of RNase (see below) and 0.5% bentonite The homogenate was centrifuged at 27000 *g* for 20 min. The sedimented membrane-bound polysomes (Palmiter 1974)

skeletal muscle and kidney convert exogenous AA into PGs of the D_2 E_2 $F_{2\alpha}$ and I_2 series The relative distribution of the PGs formed is not the same in the forearm and in the kidney indicating the existence of tissue specificity in their PG biosynthesis Furthermore the results demonstrate the occurrence of considerable inter individual variations in the quantitative relations between the various PGs formed In the light of the pronounced and varying biological effects of different PGs the present data demonstrating tissue specificity and interindividual differences in formation support the hypothesis that PGs may contribute to physiological regulatory processes in human tissues

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Ribonuclease activities in developing experimental granulation tissue with reference to polysomes

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AHO S. & KULONEN E. Ribonuclease activities in developing experimental granulation tissue with reference to polysomes. Acta Physiol Scand 1979 106, 313–318. Received 15 Jan 1979 ISSN 0001-6772. Department of Medical Chemistry University of Turku, Finland

The Mg^{2+}-precipitable polysomes in experimental granulation tissue increase up to three weeks and decrease slowly thereafter. Polysomes from young granulation tissue are on a weight basis, more active in cell-free protein synthesis than polysomes from old granulation tissue. RNase activity is highest in polysomes from 1-week tissue. The polysomes contain both acid and alkaline RNase but the alkaline RNase is in latent form manifested by the addition of p-chloromercuribenzoate (pCMB). The high RNase activity of granulation tissue renders the analysis of polysomal patterns and functions of the polysomes difficult. In the post-polysomal supernatant of granulation tissue the RNase activity is highest in 4 week old tissue; it is maximal at pH 7–8 and only slightly influenced by pCMB.

Key words: Development, granuloma, polysomes, ribonuclease

In developing granulation tissue cell proliferation and the synthesis of nucleic acids, hyaluronate and cell proteins is followed by the synthesis of collagen and chondroitin sulphate and, finally, involution (Lampiaho & Kulonen 1967, Kulonen 1970). In this work we have investigated the RNase activities of the granulation tissue and related them to the RNA content and the capacity of the tissue for protein synthesis.

The cellular protein synthesis and hence the cell proliferation is indirectly controlled by the level of cellular RNA, altered by changes in either synthesis or degradation. The RNase inhibitor from liver supernatant stabilizes polysomes during the isolation (Bont et al. 1965, Gribnau et al. 1969). The cellular RNase/RNase-inhibitor system is thus involved in the regulation of protein synthesis (Kraft & Shortman 1970a, Lan et al. 1975, Liu & Matrisian 1977, Blackburn et al. 1977).

MATERIAL AND METHODS

Experimental granulation tissue. Granulation tissue was induced by subcutaneous implantation of viscose cellulose sponge into 3 month-old female albino rats (Viljanto & Kulonen 1962). Two or three weeks after the implantation the granulomas were removed. They were processed fresh and the preparations stored frozen.

Isolation of the polysomes for protein synthesis and RNase assay. Polysomes were isolated with Mg^{2+}-precipitation (Palmiter 1974, Aho & Kulonen 1977). 1 g of tissue was homogenized into 5 ml of 25 mM tris-buffer pH 7.5 containing 25 mM NaCl, 5 mM $MgCl_2$, 1 mg/ml heparin and 2% Triton X 100. The homogenate was centrifuged at 27000 g for 10 min, after which the supernatant was diluted with 4/5 vol. of the homogenization buffer plus 1/5 vol. of 1 M $MgCl_2$, and incubated at 0°C for 1 h. The precipitated polysomes were collected by similar centrifugation through 10-ml cushion of sucrose (10% sucrose 25 mM tris-buffer pH 7.5 25 mM NaCl 5 mM $MgCl_2$). After the centrifugation the supernatant was removed by aspiration, the upper part of the tube was washed with water which was removed by aspiration, and the sucrose layer was decanted off. The walls were wiped dry and the pellet was suspended in water to the concentration of 90 A_{260}-units/ml and stored in liquid nitrogen.

Preparation of the polysomes for the sedimentation profiles. About 15 g of 3-week-old granulation tissue was homogenized with the Ultra-Turrax homogenizer (Janke & Kunkel, Staufen i. Breisgau, Germany) into 10 ml of medium, which contained one part 90 mM tris-buffer pH 7.4, 10 mM magnesium acetate, 1 M KCl and 120 mM sucrose plus one part of the liver supernatant prepared according to Gagnon & de Lamirande (1973) for the inhibition of RNase (see below) and 0.5% bentonite. The homogenate was centrifuged at 27000 g for 20 min. The sedimented membrane-bound polysomes (Palmiter 1974)

skeletal muscle and kidney convert exogenous AA into PGs of the D_2 E_2 $F_{2\alpha}$ and I_2 series The relative distribution of the PGs formed is not the same in the forearm and in the kidney indicating the existence of tissue specificity in their PG biosynthesis Furthermore the results demonstrate the occurrence of considerable inter individual variations in the quantitative relations between the various PGs formed In the light of the pronounced and varying biological effects of different PGs the present data demonstrating tissue specificity and interindividual differences in formation support the hypothesis that PGs may contribute to physiological regulatory processes in human tissues

This study was supported by grants from the Swedish Medical Research Council (project 14X-4341) and from Förenade Liv Mutual Group Insurance Company Stockholm Sweden 13 14-dihydro-15-keto-PGE_2 PGD_2 $PGF_{2\alpha}$ PGE_2 and PGI_2 were kindly supplied by Dr J Pike (Upjohn Company)

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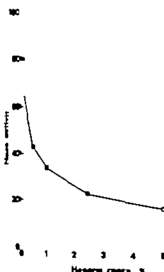


Fig. 2. Heparin inhibition of the ribonuclease activity in the granulation tissue extract. Two granulomas were homogenized in 5 ml of 0.2 M tris-buffer pH 7.8, 1.5 mM $CaCl_2$. The homogenate was centrifuged at 20 000 g for 30 min and the ribonuclease activity in the 20 000 g supernatant was determined as described in the Experimental section. In the reaction mixture the granulation tissue extract was diluted 1 : 225.

There are several ways of explaining the absence of the 100 S fraction in the preparations from the granulation tissue: (1) A very low initiation frequency in translation in the granuloma would cause only a few ribosomes to become attached to mRNA. (2) Ribosomes may have loosened from mRNA during the homogenization of the tissue and isolation of polysomes. (3) The homogenization of the tissue or (4) RNase action may have broken the mRNA into fragments, each carrying only a few ribosomes. The fact that the first supernatant (Fig. 1, left) of the granulation tissue contains still some large polysomes points to the RNase effect in the granulation tissue. During the Mg^{2+}-precipitation of the polysomes (Fig. 1 right) the ribonuclease could have broken the mRNA, resulting small polysomes (135 fraction) to be seen in the sucrose gradient.

Heparin is generally used as RNase inhibitor during the isolation of polysomes from various tissues. The RNase activity in granulation tissue supernatant needs abnormally high heparin concentration to be totally inhibited (Fig. 2). In the presence of 1% heparin granulation-tissue extract diluted in the ratio 1 : 225 retains 66% of its original ribonuclease activity. We therefore applied other RNase inhibitors to the granulation tissue extract. Liver supernatant and 250–500 mM KCl were quite effective inhibitors for alkaline RNase (inhibition 61% and 91% respectively). Bentonite (0.5% w/v) inhibited RNase in 1 : 3 dilution of granulation tissue supernatant (2 g/5 ml) by 53% and the liver supernatant (one-third v/v) by 92%. Diethylpyrocarbonate was not inhibitory at the concentration of 1.66% (v/v).

Polysomes from granulation tissue incorporate ^{14}C proline into protein in the wheat-germ cell-free system. Aurintricarboxylic acid (0.01 mM), which inhibits protein synthesis at the initiation, had no effect. Emetine (1 μM), which is an inhibitor of the elongation, reduced the incorporation by half. In the presence of 50 μg of pancreatic ribonuclease A there was no protein synthesis.

These results support the interpretation that the granulation tissue RNase breaks the mRNA into fragments carrying only a few ribosomes each. Nascent peptides on ribosomes are elongated further but there is no more initiation of new peptides in the cell-free system.

Ribonuclease activity during the development of the granulation tissue

The low polysome content in one week-old granulation tissue increases rapidly to reach a maximum in three week-old tissue; thereafter it decreases slowly (Fig. 3). The polysomes from young tissue are very active in cell-free protein synthesis compared

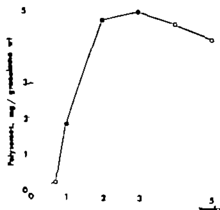


Fig. 3. Polysome yield from developing experimental granulation tissue of 1–5 weeks.

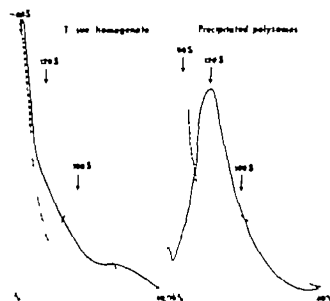


Fig 1 Distribution of polysomes from the experimental granulation tissue relative to rat liver polysomes Tissues were homogenized polysomes isolated and the profiles analyzed in sucrose gradients as described in the Experimental section (*Left*) Tissue homogenate free polysomes from the granulation tissue (——) and from liver (– – –) obtained without detergents (*Right*) Precipitated poly somes from the granulation tissue (——) and from the liver (– – –)

were suspended in 10 ml of the same medium which contained detergent (1% Triton X 100 and 0.2% sodium dodecyl sulphate SDS) but no betonite The homogenate was again centrifuged at 27 000 *g* for 20 min The sedimentation profile of the free polysomes was determined from the first 27 000 *g* supernatant and that of the membrane-bound polysomes from the second 27 000 *g* supernatant as described below

A sample of rat liver was homogenized with a Potter Elvehjem homogenizer into medium containing 20 mM tris-buffer pH 7 4 240 mM KCl 7.5 mM $MgCl_2$ 170 mM sucrose plus 0 1% heparin (Oy Star Ab Tampere) and 0 % yeast RNA The homogenate was centrifuged first at 1 100 *g* for 10 min and the supernatant then at 27 000 *g* for 20 min. The sedimentation profiles of liver polysomes were determined from the 27 000 *g* supernatant

Sedimentation profile of polysomes Samples of 1 ml from the 27 000 *g*-supernatants, corresponding to 0 15 g wet weight of granulation tissue or 100–200 µl (5–10 A_{260}-units) of the suspension of the isolated polysomes were layered over linear gradients of 14 ml of 15–40% sucrose in 20 mM tris-buffer pH 7 4 containing also 240 mM KCl and 7.5 mM $MgCl_2$ After centrifugation for 75 min at 100 000 g_{max} in the MSE 65 ultracentrifuge (6×15 ml swing-out rotor) the gradients were pumped through a flow cell with a 1-cm light path. The absorbance at 260 nm was measured with a Perkin-Elmer UV VIS 139 spectrophotometer attached to a Servogor RE 514 9 recorder

Preparation of the liver supernatant used as the RNase inhibitor The liver supernatant was prepared as described by Gagnon & de Lamirande (1973) Livers from four rats were homogenized with a Potter-Elvehjem homogenizer in 100 ml of 50 mM tris-buffer pH 7.6, containing also 25 mM KCl 10 mM magnesium acetate, 10 mM mercaptoethanol and 350 mM sucrose. The homogenate was centrifuged at 27 000 *g* for 15 min and the supernatant for a further 2 h at 100 000 *g* The 100 000 *g* supernatant was divided into aliquots which were stored at −70°C

Protein synthesis with granulation tissue polysomes Polysomes from the granulation tissue were prepared as described above The S-30 supernatant from wheat-germ was prepared according to Roberts & Paterson (1973) The cell-free protein synthesis was carried out as described earlier (Aho & Kulonen 1977), except that the deacylated tRNA from the granulation tissue was omitted from the reaction mixture

Determination of the RNase activity The RNase activity in tissue or polysome samples was determined in a final volume of 300 µl which contained 50 µl of 1 M tris-HCl pH 7 8 50 µl of 0.02% bovine serum albumin (Sigma, cat No A-4378) and 50 µl of yeast RNA-solution. The yeast RNA (Sigma, type II Cat No R-6500) was dialyzed for 24 h against 10 mM EDTA for a further 24 h against 0 1 M NaCl and finally for 24 h against water The concentration was adjusted to 2% (w/v) on the basis of UV-absorption (21 A_{260}-units correspond to 1 mg RNA/ml). Tissue supernatants and polysomes were usually added in 100 and other ingredients in 50 µl of solution. For the assay of the total RNase activity the reaction mixture was the same except that 50 µl of 4-mM *p*-chloromercuribenzoate pCBM (Fluka) in 0.02% bovine serum albumin was added instead of the plain albumin solution The reaction mixtures were incubated in a shaking water bath at 37°C for 1 h at which time the tubes were placed in an ice-water bath. After 1 min an equal volume (300 µl) of cold 75% (v/v) ethanol in 1 M HCl was added to stop the reaction and the samples were chilled for 1 h to complete the precipitation The blank assay in which the enzyme solution was added after the ethanol-HCl was carried out simultaneously and kept during the incubation in an ice-bath. The tubes were centrifuged at 3 000 *g* for min at 4°C the 500 µl sample of the supernatant was diluted with 500 µl of water and the increase in the absorption at 260 nm was measured

RESULTS

Polysome distribution and protein synthesis in granulation tissue

The homogenization of the granulation tissue with the Ultra Turrax homogenizer detaches the polysomes from the membranes and no polysomes are left to be liberated by the detergents (Fig. 1 left). During the isolation process the granulation-tissue polysomes are broken into mono- di and trisomes (120S fraction Fig 1 right) In the corresponding preparations from the liver there appear distinct peaks of large polysomes (180S and larger)

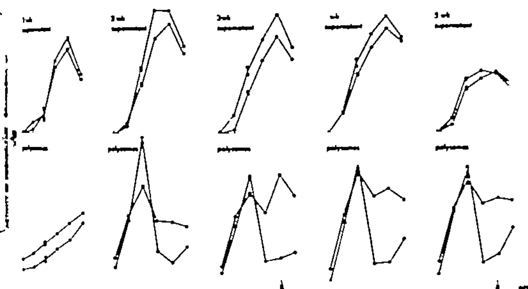


Fig. 6. The pH dependence of RNase activity in post-polysomal supernatant and polysomes from granulation tissue at various developmental stages. Succinate-borate buffer (0.2 M) was used between pH 4.0 and 6.0 and phosphate-borate buffer (0.2 M) between pH 6.0 and 9.0. RNase activity: μmol nucleotides liberated/h/reaction mixture. O—O free RNase, ●—● RNase activity with pCMB.

The post-polysomal supernatant from the granulation tissue contains an active alkaline RNase only slightly inhibited by pCMB. The activity of this RNase is highest in three week-old granulation tissue (Figs. 6 and 7) when the collagen synthesis is maximal. McIntosh & Rabin (1975) found that rat liver post-microsomal supernatant contains a minor RNase activity which could not be inhibited by the protein inhibitor of RNase, but instead by pCMB. They report that the autodegradation of rRNA in the preparations of whole microsomes is due to this RNase. Gilmartin & Freedberg (1976) found a deficiency of initiation in protein-synthesizing systems prepared from mammalian epidermis, which may be due to prior partial degradation of ribosomal subunits or messenger RNA by RNase. The system remains, however, sensitive to the elongation inhibitors and the exogenous RNase.

Von Tigerstrom & Manchak (1977) investigated the heterogeneity of the acid- and heat-stable alkaline RNase, which is found in most mammalian tissues and body fluids. This heterogeneity may be due to differences in the polysaccharide moieties in the same protein molecule, which may cause variation, for example, in the sensitivity to RNase inhibitor. Differences in the oligosaccharide part of RNase have also been described by Baynes & Wold (1976).

The physiological role of this heterogeneity remains to be explored. It could be connected with the differentiation of the cellular composition and of synthesis of the various proteins in the tissues.

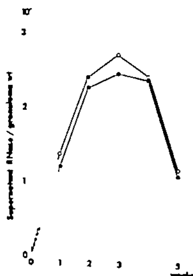


Fig. 7. Alkaline RNase (pH 7.8) content of post-polysomal supernatant from granulation tissue. RNase activity: μmol nucleotides liberated/h/reaction mixture. O—O free RNase, ●—● with pCMB.

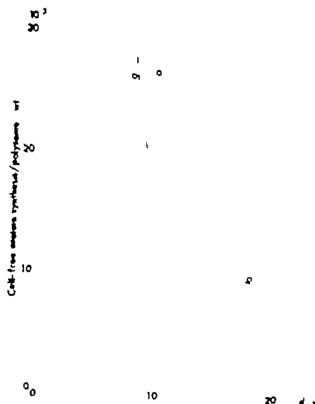


Fig 4 The capacity of granulation tissue polysomes for cell free protein synthesis

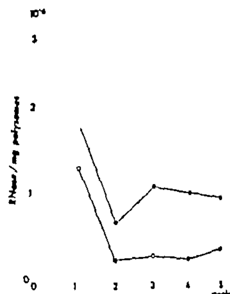


Fig 5 Alkaline RNase (pH 7.8) content of polysomes from granulation tissue RNase activity μmol nucleotide liberated/h/reaction mixture O—O free RNase, ●—● RNase activity with pCMB

with polysomes from older tissue (Fig 4) This may be due to the predominance of non fibroblast cells in the tissue during the early phase Polysomes from one-week-old granulation tissue contain a high specific activity of alkaline RNase This activity decreases in older granulation tissue polysomes which contain alkaline RNase in latent form (Fig. 5)

In Fig 6 there is shown the pH dependence of RNases from the polysomes of the granulation tissue Polysomes from one week-old granulation tissue contain no acid RNase at all but an RNase with a very alkaline pH-optimum Polysomes from 2–5-week-old granulation tissue contain both an acid and an alkaline RNase but the latter is in latent form bound to an endogenous inhibitor The amount of this latent RNase is highest in three week-old granulation tissue This result is in agreement with earlier findings (Jalkanen et al 1978)

The post polysomal supernatant from tissue two- to four weeks old contains very active alkaline RNase (Fig. 7) which has a pH-optimum between 7 0 and 8 0 as can be inferred also from Fig 6 There is no RNase inhibitor

DISCUSSION

Our results agree with previous reports that activities of alkaline and acid RNases and RNase inhibitor are high in tissue with high levels of cell proliferation and protein synthesis (Kraft & Shortman 1970*b* Blackburn et al 1977) In developing granulation tissue there is a peak in the DNA synthesis at 7 days RNA synthesis reaches a peak at 14 days and protein synthesis at 21 days There is a low yield of polysomes in one-week-old granulation tissue and they have a rather high specific activity of free alkaline RNase Polysomes from older granulation tissue contain almost all their alkaline RNase in latent form Murthy & McKenzie (1974) and Roth (1976) found that changes in the level of total alkaline RNase and RNase inhibitor tend to stabilize the activity of free alkaline RNase the balance of RNase and RNase inhibitor reflected the proportion of polyribosomes in a polysome profile Green (1977) observed a marked decrease in acid RNase activity during lymphocyte stimulation concomitant with the accumulation of RNA The activity of acid and alkaline RNases in reticulocytes is only one 30th to 50th of that in bone marrow erythroid cells (Hulea & Arnstein 1977) The most marked decrease in RNase activity of the reticulocytes occurred in the fractions containing ribosomes, mitochondria and lysosomes

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Muscle strength and muscle characteristics in monozygous and dizygous twins

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KARLSSON J., KOMI P. V. & VIITASALO J. H. T. Muscle strength and muscle characteristics in monozygous and dizygous twins. Acta Physiol Scand 1979 106: 319–325. Received 17 Jan. 1978. ISSN 0001-6772. Laboratory for Human Performance (FOA 57), Karolinska Hospital, Stockholm, Sweden and Kinesiology Laboratory, University of Jyväskylä, Finland.

Muscle strength and electrical activity were investigated on 31 pairs of young male and female monozygous (MZ) and dizygous (DZ) twins. The measurements included leg forces, force-time, running velocity, muscular power, maximal integrated electromyographic activity (IEMG) and chronaximetry of the quadriceps muscle group. In each parameter the intrapair variance was computed and the differences were tested between the MZ and DZ twins. The variance ratio (MZ vs. DZ) was statistically significant only for muscular power confirming an earlier finding which has demonstrated a genetic component for the variable. In addition to the various performance variables several key enzymes involved in ATP turnover during muscle contraction and in glucose residue metabolism were analyzed from the muscle biopsy samples (m. vastus lateralis). A genetic component could not be observed in any of their activities or their relationships to performance variables.

Key words: Strength, anaerobic performance, muscle enzymes, electromyography, heredity

Man's physical performance capacity is determined primarily by genetic and environmental factors. A major question arises to what extent these two components have an effect upon a particular trait of the physical performance measurements. Studies conducted on monozygous (MZ) and dizygous (DZ) twins have suggested the impact of the genetic component on the interindividual variation observed in maximal oxygen uptake and muscular power (Klissouras 1971, Komi et al. 1973). The twin study concept as developed by Holzinger (1929) has also been applied to human skeletal muscle and its histochemical and biochemical properties (Komi et al. 1977) and it was shown that the hereditary factors determined almost solely the variance in skeletal muscle fibre composition. The present study was undertaken to investigate on these same MZ and DZ pairs of both sexes the genetic factors involved in muscle strength performance and related muscle characteristics.

METHODOLOGY

Twins

Subjects for the study were obtained through the Population Register of Finland. The final sample was composed of 20 male (9 MZ and 11 DZ) and 11 female (6 MZ and 5 DZ) twin pairs in the age group 11–24 years. Determination of zygosity was performed by subjective observation of physical appearance and serological analyses. In the 16 cases of dizygosity discordance was observed in more than five antigens or serum proteins (Komi et al. 1977).

Maximal muscular power was determined using the method of Margaria et al. (1966). In this measurement the subject ran maximally for a few seconds in a staircase. The running velocity (V) was measured electronically and converted to the vertical component (V_v). Mechanical power kgm/s was computed on the basis of the subject's V_v and his body weight. This test is referred to as an "anaerobic test" (Margaria et al. 1966).

Muscular forces were measured as maximal voluntary isometric extensions of the right leg (quadriceps force) and of both legs (total leg forces), respectively. The type of dynamometers and testing specifications employed have been reported elsewhere (Komi 1973, Komi & Viitasalo 1975).

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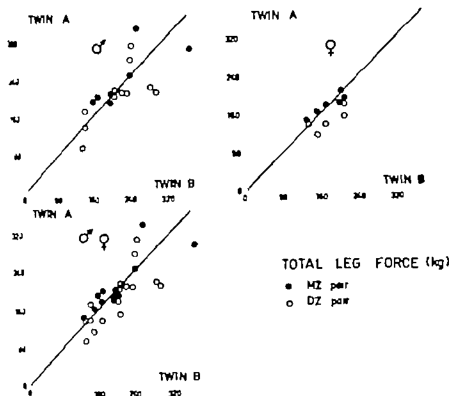


Fig. 3. Intrapair comparison of total leg force for MZ and DZ twins

[illegible] Packard 2116C computer system (Viitasalo & [illegible] 1975)

Chronaximetric measurements. Neuroton Model 626 [illegible] was used to investigate the vastus lateralis [illegible] with constant current method using the following [illegible] durations: 0.1 ms, 1 ms and 30 ms. The 30 ms [illegible] was considered as a rheobase stimulus and was [illegible] used as a basis for calculation of the chronaxie [illegible].

Skeletal muscle fibre composition and muscle enzyme [illegible] were determined in muscle biopsy samples taken [illegible] the vastus lateralis muscle and analyzed according to [illegible] et al. (1972) and Lowry & Passonneau (1972). [illegible] types were identified as slow twitch (ST) and fast [illegible] (FT) fibres. For details see Komi et al. (1977). The [illegible] enzyme activities were analyzed: creatine [illegible] (CPK), myokinase (MK), Ca^{++} and Mg^{++} stimulated [illegible] (Ca^{++} ATPase, Mg^{++} ATPase), hexokinase [illegible], phosphorylase (Phos) and lactate dehydrogenase [illegible].

[illegible] analysis. Ordinary statistical procedures [illegible] employed to calculate means, standard deviation (S.D.) and linear correlation coefficients ( ), and to test the significance of differences in intrapair variances between the two twin types. If the variance ratio (F) was significant at 5% level of probability, heritability estimate (H_{est}) was computed as originally described by [illegible] (1929)

$$H_{est} = \frac{S^2DZ - S^2MZ}{S^2DZ - S^2}$$

where S^2MZ and S^2DZ denote intrapair variability of an attribute in MZ and DZ twins, respectively, and S^2 signifies the variance due to experimental error. For details see Komi et al. (1977).

RESULTS

Hereditary factors

Functional tests. The intrapair variance for muscular power was significantly smaller in MZ pairs as compared to DZ pairs. The calculated H_{est} was 97.8. For the remaining functional tests no statistically significant difference within DZ pairs as compared to MZ could be demonstrated, but with the exception of quadriceps force the variation was slightly greater within DZ pairs (Figs. 1–4).

IEMG and chronaximetric tests. The general finding from the different IEMG and chronaximetric data was that the variation between MZ twins was slightly, but not significantly, smaller than the variation between DZ twins (Fig. 5).

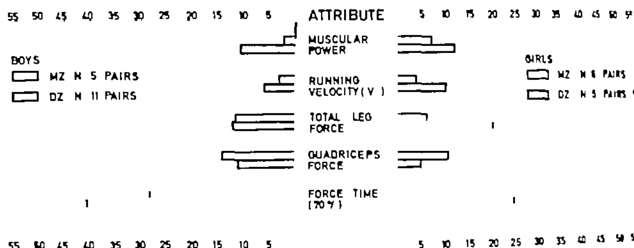


Fig 1 Mean percent intrapair difference in MZ and DZ twins for various performance data

Force time curve was registered during the total leg force measurements and the time to reach 70% of the maximum force was taken as a force-time value

Integrated electromyographic activity (IEMG) was obtained from both m rectus femoris and m vastus lateralis during maximum right knee extension w[illegible] Beckman miniature sized surface electrodes and amplif[illegible] with Brookdeal 9432 preamplifiers. After storage of [illegible] data on magnetic tape (Philips Analog 7 tape recorder) [illegible] processing of the EMG signals was performed [illegible]

Fig 2 Intrapair comparison of maximal muscular power for MZ and DZ twins

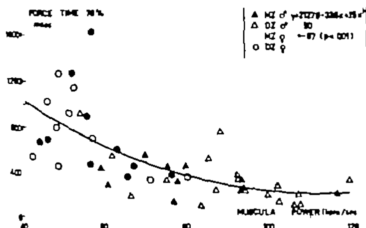


Fig. 6. Relationship between value of force-time at 70% of maximum isometric contraction (total leg force measurement) and maximal muscular power for all twin groups.

Correlation analysis

Linear correlations between all the variables studied have been published elsewhere (Komi & Karlsson 1978). In the following only the most relevant information is given.

Muscular power was in the whole material as expected, best related to other strength variables as quadriceps force ($r=0.82$), total leg force ($r=0.71$) and running velocity ($r=0.67$) as well as force-time ($r=-0.67$, Fig. 6). No significant relationship was, however, present between muscular power and per cent of ST fibres.

Considering zygosity similar relationships were obtained for muscular power versus other strength performance variables as in the whole material. The DZ twins were the only group, however, to demonstrate correlations to total leg force ($r=0.76$) and EMG of the vastus lateralis muscle ($r=0.46$).

Quadriceps force was in the whole population best related to muscular power ($r=0.82$), total leg force ($r=0.80$) and force-time ($r=-0.46$). No differences in terms of correlations seemed to be present for quadriceps force when comparing monozygous (mean value 79±4 kp) and dizygous twins (mean value 5 ±16 kp). In the males quadriceps force was also related to per cent ST fibres ($r=-0.55$).

Activities of enzymes involved in ATP turnover during muscle contraction. Among the enzymes studied only CPK demonstrated in the whole population correlations to strength performance variables, running velocity ($r=0.41$) (Fig. 7) and force time ($r=-0.40$) respectively. The enzyme activities and their relations to performance variables were not influenced by zygosity.

Activities of enzymes involved in glucose residue metabolism. Hexokinase (HK), phosphorylase (Plase) and lactate dehydrogenase (LDH) were the enzymes selected to represent the glucose residue metabolism. They demonstrated no significant or vague relationships to performance variables. Per cent distribution of LDH 1 isozyme showed a correlation only in the MZ group versus a strength variable (muscular power, $r=-0.62$) in contrast to the DZ group as well as the whole material.



Fig. 7. Relationship between the activity of creatine phosphokinase (CPK) enzyme (moles g^{-1} min^{-1} 10^{-6}) and running velocity (m/s) on staircase.

TWIN A ♂ TWIN B

TWIN A ♀ TWIN B

TWIN A ♂+♀ TWIN B

QUADRICEPS FORCE (kg)
● MZ pair
○ DZ pair

Fig 4 Intrapair comparison of quadriceps force for MZ and DZ twins

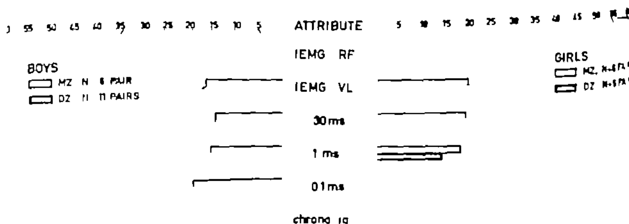


Fig 5 Mean percent intrapair differences in MZ and DZ twin for integrated electromyographic (IEMG) data from rectus femoris (RF) and vastus lateralis (VL) muscles and chronaxumetric data of different stimulus durations (30 and 1 ms 0 1 ms) from the vastus lateralis muscle

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DISCUSSION

An earlier report (Komi et al 1977) with the same twin material had observed the strong heritability estimate for the ST fibre distribution The results of the present report support earlier findings (Komi et al 1973) that also variance in muscular power is genetically determined and that muscular strength and IEMG did not show heritability in the same statistically significant way as muscle fibre composition or muscular power In these parameters a statistical comparison showed that the intrapair variance was not statistically significant at the required confidence level of $p<0.05$ For this reason the computation of H_{est} was abandoned

No special control of the socio-economic health or physical activity status was made in the present study Although these environmental influences probably have been only minor they still might be the cause of individual adaptation to some other strength performance thus masking a genetic component present Adaptation of similar kinds might affect both neuromuscular functions and muscle enzyme activities

In the present study muscle performance has been interpreted from measurements of muscular power muscular forces force-time and IEMG during maximal isometric knee extension In addition chronaximetric data were obtained from the vastus lateralis muscle Muscular power as measured in the present study is sometimes referred to as an "anaerobic power" test (Margaria et al 1966) which would imply that it would indicate the magnitude of the available ATP and CP stores as well as the potential of the anaerobic glycolytic pathway (Karlsson 1971) Although this test easily differentiates power athletes from endurance athletes (Komi et al 1977) it is the authors opinion that this hypothesis has not been satisfactorily tested

In both twin materials positive correlation coefficients were obtained however for muscular power versus lean body mass corresponding to 0 91 (MZ twins) and 0 97 (DZ twins) This is indicative that muscular power is directly related to the muscle volume The individual anthropometric variables have earlier been demonstrated to be dependent on genetic factors (Komi et al 1973) These considerations would then support the original suggestion by Margaria et al (1966) that muscular power reflects quantitatively the anaerobic energy output

Thorstensson and co-workers have in a number of studies tried to evaluate how muscle strength training is affecting muscle fibre composition and muscle enzyme activities and found no or small changes in spite of marked increases in performance (for reference and summary see Thorstensson 1976) It was suggested that the neuromotoric control was relatively more affected although no experimental evidence could be found for this hypothesis

When employing the isokinetic technique for measurements of dynamic muscle strength, it could be demonstrated that the potential of a muscle to produce force during movements of high velocity was positively correlated to its proportion of FT muscle fibres (Thorstensson 1976) Similarly it has been shown that the time needed for production of submaximal force levels is related to the muscle fibre composition (Viitasalo & Komi 1978). It seems then reasonable to suggest that even muscular strength performance might be partly under the influence of genetic factors This suggestion is basically motivated by the strong heritability estimate found for the muscle fibre composition Additional support for not rejecting this hypothesis comes from a comparatively strong correlation between muscular power and other strength variables Therefore it is encouraged that the problem will be re-investigated with a larger twin material than what was possible in the present study

The study was supported by grants from the Swedish Medical Research Council (project No 4251)

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Changes in penile volume during some cardiovascular reflexes and reactions in rabbit

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SJÖSTRAND N O & KLINGE E Changes in penile volume during some cardiovascular reflexes and reactions in rabbit *Acta Physiol Scand* 1979 106:327-334 Received 17 Jan 1979 ISSN 0001-6772. Department of Physiology Karolinska Institutet, Stockholm Sweden, and Department of Pharmacology University of Helsinki, Finland

The effect of various cardiovascular reflexes and reactions on the plethysmographically recorded penile volume in rabbit was investigated Stimulation of the aortic nerve or direct stimulation of the carotid sinus produced increase in penile volume, while carotid occlusion produced decrease Brief volume load or protoveratrine given into the right atrium produced increase in penile volume Asphyxia, hypercapnia, hypoxia, cyanide and lobeline produced decrease in penile volume, while hypocapnia increased it. Moderate blood taps decreased penile volume. With the exception of the response to asphyxia all these reactions required intact vasomotor nerves Clonidine increased penile volume if vasomotor nerves were intact but decreased it if the penis was decentralized. Penile volume decreased in shivering animals but increased on warming Carotid occlusion impaired erectile responses to hypogastric and pelvic nerve stimulation. In certain experiments this effect was more pronounced in the latter case It is concluded that the medullary neuron pool responsible for penile vasomotor tone participates in general reflex cardiovascular homoeostasis and that this may have implications for normal erectile responses

Key words Penis, erection cardiovascular reflexes, vasodilation, smooth muscle

In a preceding paper (Sjöstrand & Klinge 1979) we reported that during the flaccid state of the rabbit penis the organ was governed by an adrenergic vasomotor tone of strikingly low intensity During the course of the experiments summarized in that report we noted—somewhat to our surprise—that the penile volume changed easily and quite markedly when standard cardiovascular reflexes were elicited. This suggested that the central neuron pool responsible for penile vasomotor tone participates in reflex cardiovascular homoeostasis in a way quite similar to the central pools controlling the tone of the resistance vessels of skeletal muscle One could have expected the penile vasomotor nerves, operating at very low frequencies to behave more like those of the pad and not to participate readily in e.g. baroreflexes (vide e g. Folkow & Neil 1971 Öberg 1976)

In this study we present data showing that the penile vascular bed as evident from plethysmographical recordings of penile volume participates in several general cardiovascular reflexes and reactions These findings will be discussed with respect to the special function of this unique vascular bed which has an anatomically dual but physiologically as yet obscure *vasodilator innervation* i.e. a parasympathetic as well as a sympathetic dilator innervation with indefinite neurotransmitters (cf Sjöstrand & Klinge 1979)

MATERIAL AND METHODS

Animals, anesthesia, preparation procedures, care equipment and most drugs have been described in the preceding report (Sjöstrand & Klinge 1979) Here some special considerations will be added.

In a few expts. the urethan anesthesia was replaced by sodium pentobarbital (120-200 μmol/kg i.) or α-chloralose (100-160 μmol/kg i.) supplemented with small doses of urethan. Concerning the studied reflexes these kinds of anesthesia represented no advantage.

Arterial stretch receptor reflexes. Centripetal stimulations (4-6 ms, 15-35 V) of the cut aortic nerves were

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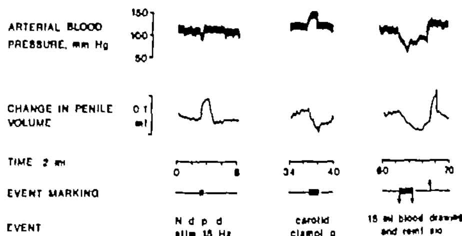


Fig 1 Rabbit 3 0 kg urethan blood pressure recorded from left carotid artery Both aortic nerves and left vagus nerve cut Left panel Centripetal stimulation of right aortic nerve produces drop in blood pressure and increase in penile volume Middle panel Clamping of right carotid artery produces increase in blood pressure and reduction in penile volume Right panel 15 ml of blood is slowly withdrawn from left femoral artery Blood pressure and penile volume decrease When the blood which has been kept warm is rapidly reinfused (10 s) into the right atrium there is an immediate increase in penile volume which later stabilizes as also does the blood pressure

performed with similar electrodes as used for other nerve stimulations (Sjöstrand & Klinge 1979) Mechanical stimulation of the carotid sinus was usually performed by tugging a thread tied around the common carotid artery immediately below the bifurcation The artery was severed below the knot and the caudal part was used for blood pressure recording The carotid sinus was carefully exposed before noradrenaline was dripped on it (cf Landgren Neil & Zotterman 1952) Clamping of the common carotid artery was performed 5–15 mm below the bifurcation Usually the aortic nerves and often also the chief vagal trunks were cut when carotid sinus reflexes were studied

Cardiac receptor reflexes A venous catheter was pushed to the level of the right atrium (cf Öberg 1964) Via this catheter the receptors were excited either mechanically by rapid volume load (5–20 ml of body warm saline Ringer 6% dextran in saline or blood cf e g Kappagoda Linden & Mary 1977) or pharmacologically by protoveratrine (cf e g Jarisch & Zotterman 1948 Öberg 1964)

Respiratory reflexes and reactions These were usually provoked in rabbits kept on artificial ventilation after paralysis of the respiratory muscles by decamethonium The rabbits were subjected to hyperventilation hypoventilation or asphyxia Via the inlet of the Harvard ventilation pump the rabbits were given 6.5% CO_2 in O or 10% O_2 in N_2 The latter mixture was sometimes followed by 100% O_2 Peripheral chemoceptors were also stimulated by cyanide given either as slow (0 5–6 ml/min) i v infusion of 15×10^{-4} M KCN or rapid i v infusion of 0 1–1 ml of 2×10^{-4} M NaCN or lobeline (0.7 1 4 µmol/kg) In some rabbits Mayer waves (cf Schweitzer 1945) were provoked by bleeding (Anderson Kenney & Neil 1950) pharmacological blockade of central chemoceptors by chloralose (15–30 µmol/kg i v) (Euler & Söderberg 1952) and central pharmacological elimination of baroreflexes by ergotamine (0 7 1 4 µmol/kg i v) (Euler & Schmiterlöw 1944)

Removal of blood was performed by slow withdrawal of 10–30 ml through a catheter inserted into a femoral or brachial artery

Clonidine was infused i v using a standard dose of µmol/kg In rabbit clonidine stimulates peripheral α adrenoceptors in resistance vessels but reduces vasomotor tone due to action on the brain stem or more centrally It also produces a central facilitation of the baroreceptor cardiodepressor reflex (cf Tangri et al 1977)

Thermoregulatory responses were produced in rabbits kept on rather light urethan anesthesia i e they shivered unless the front and back of the thorax were warmed by lamps The responses were elicited by switching heat on and off One precision thermometer was placed into the rectum and another outside the plethysmograph The recorded temperature levels were usually not allowed to change during experimentation In of the expts where the effect of temperature on penile volume was studied all superficial penile vessels were tied in order to reduce the influence of an increased blood content and flow in these vessels on the volume recording

Pelvic and hypogastric nerve stimulations were performed as described by Sjöstrand & Klinge (1979).

Drugs Apart from the drugs listed by Sjöstrand & Klinge (1979) the following drugs were used, α-chloralose (ACO) clonidine hydrochloride (Boehringer Ingelheim) ergotamine bitartrate (Gynergen® Sandoz) lobeline hydrochloride (Sandoz) and protoveratrine (Sandoz)

RESULTS AND COMMENTS

General

Reflex changes in penile volume were as a rule best studied in the beginning of an expt With the excep-

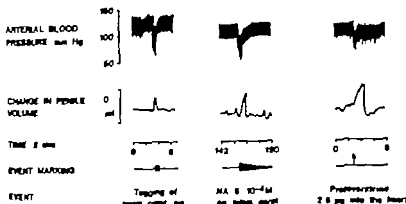


Fig 2 Left and middle panels, rabbit, 2.4 kg, urethan, blood pressure from left carotid artery. Both vagi and aortic nerves cut. Left panel: Traction of left carotid sinus decreases blood pressure and increases penile volume. Middle panel. Right carotid artery clamped. Artificial respiration after decamethonium. High concentration of noradrenaline dripped on both prepared carotid sinus induces pressure drop and penile volume increase. Right panel. Rabbit 2.8 kg, urethan, blood pressure from left carotid artery. Protoveratrine injected into right atrium produces drop in pressure and increase in penile volume.

tion of the response to asphyxia, all reflexes and reactions described below had the following in common (1) They were uninfluenced by section of the hypogastric and/or the pelvic nerves hence the penile vasodilator nerves did not participate (2) They were abolished by acute or previous section of the sympathetic chains (L 6–S 1) and there fore were due to changes in adrenergic penile vasomotor tone (cf. Sjöstrand & Klinge 1979)

Arterial baroreceptor reflexes (Figs 1 and 2)

Centripetal stimulation (5–25 Hz, 5–30) of the severed right or left aortic nerve produced increase in penile volume concomitantly with the classical depressor response (Fig. 1) Also direct stimulation of the baroreceptors as produced by mechanical stretching of the wall of the carotid sinus or contraction of its smooth muscle by dripping a noradrenaline solution on it induced increase in penile volume and drop in blood pressure (Fig. 2) On the other hand, unloading of the arterial stretch receptors caused by clamping of the common carotid artery produced decrease in penile volume together with the classical pressor response (Fig. 1) Thus in anesthetized rabbit penile vasomotor neurons participate in high pressure side stretch receptor reflexes

Cardiac receptor reflexes (Fig 1 and 2)

Brief volume load on the right heart produced clear-cut increase in penile volume (cf Fig. 1) moderate initial drop in blood pressure and slowing of the heart. Also 2–10 µg of protoveratrine injected into the heart produced marked increase in penile volume bradycardia and moderate hypotension (Fig. 2) Section of the vagi greatly reduced the response to volume load and eliminated that to protoveratrine This suggests that penile vasomotor neurons may participate also in low pressure side stretch receptor reflexes originating in the right atrium and possibly in the ventricle

Respiratory reflexes and reactions (Fig 3)

Asphyxia caused by occlusion of the airways, acute paralysis of the respiratory muscles or interruption of artificial respiration in paralyzed animals always produced a powerful initial pressor response and a prompt retraction of the penis (Fig. 3). The penile response persisted after section of the sympathetic chains but became slower Also in rabbits in which besides this neurotomy the right adrenal was ligated and the left splanchnic nerve was cut (cf Sjöstrand & Klinge 1979) asphyxia produced retraction although it was fairly slow We therefore conclude that the penile retraction was due to increased penile vasomotor tone as well as to increased levels of circulating catecholamines derived from the adrenal medulla (cf e.g. Celander 1954) and probably also from adrenergic neurons other than those innervating the penis.

Hypercapnia caused by inhaling carbogen gas produced an initial decrease in penile volume to-

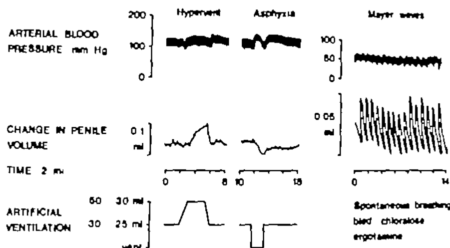


Fig 3 Left and middle panels rabbit 3 kg urethan blood pressure from left carotid artery both vagi and aortic nerves cut decamethonium artificial respiration. Left panel Doubling of ventilation produces increase in penile volume Middle panel. Interruption of ventilation induces a pressor response and reduction in penile volume Right panel. Rabbit 4 kg urethan blood pressure from right femoral artery The rabbit which had lost blood and had received chloralose and ergotamine displayed Mayer waves in blood pressure Concomitantly with the pressure increases the penile volume decreases while it increases on pressure reductions.

gether with a rise in arterial blood pressure On the other hand *hypocapnia* produced by hyperventilation led to an initial increase in penile volume (Fig. 3) The responses to hyper and hypocapnia were not eliminated by section of the aortic nerves and carotid occlusion and were therefore presumably elicited from central chemoceptors (cf e g Heymans & Neil 1958 Folkow & Neil 1971)

Stimulation of *peripheral chemoceptors* by *hypoxia cyanide* or *lobeline* produced the expected initial rise in blood pressure (cf Heymans & Neil 1958 Korner Chalmers & White 1967) Concomitantly the penile volume decreased If the penile volume was decreased by a short period of breathing 10% O_2 in N_2 it could be instantly increased by inhaling pure oxygen

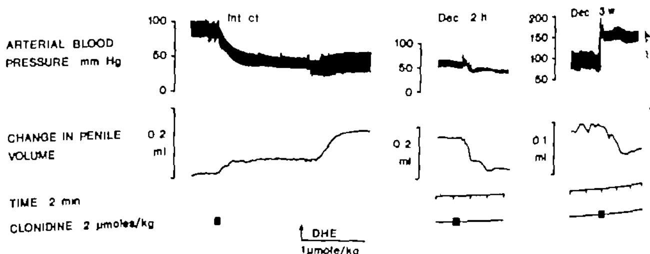


Fig 4 Effect of clonidine on penile volume and on blood pressure recorded from left carotid artery Urethan anesthesia Left panel. Intact rabbit, 2.8 kg Clonidine produces drop n blood pressure and increase in penile volume Dihydroergotamine further increases penile volume Middle panel. Rabbit 3 8 kg Sympathetic paravertebral decentralization of the penis performed at L6–S1 h prior to tracings. Iliac vessels tied Clonid'ne produces drop in blood pressure penile volume decreases Right panel. Rabbit 3 9 kg Sympathetic chains cut 3 weeks prior to expt Clonidine increases blood pressure and decreases penile volume

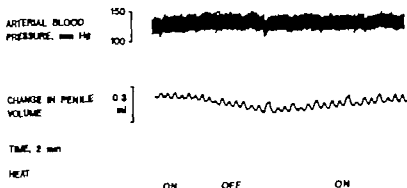


Fig 5 Rabbit, 2.5 kg, urethan blood pressure from left carotid artery Superficial penile vessels tied Penile volume shows rhythmic changes. The rabbit was warmed at the thoracic level on both front and back and was in light anesthesia. When heat was switched off the *rabbit gradually started to shiver* and penile volume decreased but it increased again when heat was turned on. During tracing the rectal and room temperatures ere constant at 34 4° and 4.3°C respectively

he participation of penile vasomotor neurons in pheral chemoceptor reflexes was further illused when Mayer waves (vide Schweitzer 1945 ierson et al. 1950, Heymans & Neil 1958) were voked by bleeding combined with chloralose l ergotamine treatment. Thus the penile volume nged rhythmically with the fluctuations in ar al blood pressure dimimshing when pressure e and increasing when pressure dropped (Fig. 3)

sponse to bleeding (Fig 1)

derate blood taps (10–30 ml) leading to moderate potension produced an immediate decrease in ile volume (Fig. 1) that was rapidly restored er reinfusion of blood Hence in the urethan esthetized rabbit penile vasomotor neurons also rticipate in the complex reflex cardiovascular adstments after bleeding (vide Sjöstrand 1976 berg 1976).

Effect of clonidine (Fig 4)

n intact rabbits the drug produced hypotension, metimes preceded by a brisk hypertension. Penile volume increased. The increase could be augmented by a small dose of the α-adrenoceptor blocking agent dihydroergotamine (Fig. 4) which by itself had a moderate retractile effect on the lens (cf. Sjöstrand & Klinge 1979) If the sympathetic chains had been cut clonidine retracted the lens In animals with chronic sympathetic decentralization of the hind body clonidine produced hypertension (Fig. 4) We conclude that clonidine has a strong direct α-adrenoceptor stimulant action on penile vessels but that in the intact rabbit the inhibitory effects on vasomotor tone dominate, i.e the penile bed follows the scheme of other vascular beds (cf Tangri et al 1977)

Thermoregulatory eactions (Fig 5)

In animals being in light anesthesia and inclined to shivering when warming was omitted penile volume decreased gradually when heat was switched off but increased on rewarming (Fig. 5) These changes in penile volume were observed before the rectal temperature was affected but became still more marked if the rectal temperature was allowed to change

Influence of the carotid occlusion eflex' on the erectile response to pelvic and hypogastric nerve timulation (Fig 6 and 7)

The vasoconstrictor fibres are powerful antagonists of the dilator fibres (cf Sjöstrand & Klinge 1979) It therefore seemed of interest to investigate whether the increased vasomotor tone due to relief of baroreceptor restraint and possibly also to increased drive from carotid bodies, which occurs after carotid clamping, could impair the erectile response to vasodilator stimulation. This was in fact the case i.e in vagotomized rabbits the erectile responses due to 20 stimulations (4–8 Hz) of the

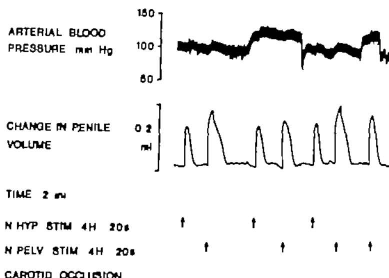


Fig 6 Rabbit 3 3 kg, urethan blood pressure from left femoral artery Vagi and aortic nerves cut The erectile response produced by stimulation of the pelvic nerves during continuous bilateral carotid occlusion is depressed whereas the erectile response elicited by stimulation of the hypogastric nerves under the same conditions is unimpaired

hypogastric or pelvic nerves were often diminished when performed *during* carotid clamping in spite of the increase in blood pressure which in itself would have led to augmented responses In some expts as in Fig 6 the pelvic response was diminished to a greater extent. Also carotid clamping started *after* elicitation of the erectile responses could have retractile effects In analogy to the effects observed at direct stimulation of the sympathetic chains (Sjöstrand & Klinge 1979) a difference between the effects on hypogastric and pelvic nerve stimulation could be noted in some experiments Thus Fig 7 shows records from an expt in which carotid clamping clearly interfered with the erectile response to pelvic nerve stimulation but not with that to hypogastric nerve stimulation

DISCUSSION

The influence of changes in environmental temperature on penile volume is a daily experience of every man From a functional point of view it seems plausible that the primary thermoregulatory event is an altered flow in the arterio-venous shunts present in the penis (Müller 1935 and later authors) In fact, thermoregulation is the most likely function of these vessels not the speculative one so often proposed i e that they close during erection and thereby direct the flow into the cavernous space (e g. Conti 1952) Participation of the inflow resistance vessels to the cavernous bodies and of the muscles of the erectile tissue in thermoregulatory reactions could be due e g to a certain coupling between the vasomotor tone of these vessels and that of the shunts Furthermore it is apparent to anyone who has watched a lone gelding on a hot summer day that also the retractor penis participates in thermoregulatory reactions The obvious advantages of participation of the smooth muscle effectors of erection in penile thermoregulatory reactions are control of the area from which heat is dissipated and control of the temperature gradient between surface and core of the organ. Naturally under more extreme conditions temperature also has direct effects on penile smooth muscle (cf. Klinge & Sjöstrand 1974)

Obviously penile vessels participate in various other cardiovascular reflexes and reactions From the point of view of general cardiovascular homeostasis we consider our findings merely academical curiosities Thus the resistance offered by the penile bed must be a diminutive fraction of the total peripheral resistance and the volume of blood received or delivered by the penis during the reactions is just some thousandths of the total blood volume However considering the function of the penis we believe that the findings deserve some attention At least they raise certain questions Firstly is there any central inhibition at all of penile vasomotor tone during a normal erection? Secondly could it not be so that penile vasomotor tone in

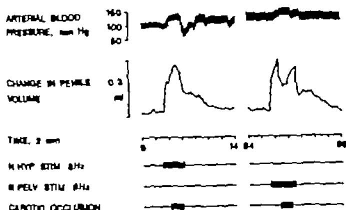


Fig. 7. Rabbit, 3.0 kg, urethan blood pressure from left carotid artery. Both vagi and aortic nerves cut. Left panel: Hypogastric nerves are stimulated. When right carotid artery is occluded the pressure increases and so does the penile volume. Right panel: Pelvic nerves are stimulated. When carotid artery is clamped there is decrease in penile volume. This rabbit had tendency to cardiac arrhythmia when carotid occlusion was relieved, as seen in the left recording.

but increases during coition, or alternatively if one assumes that penile vasomotor tone is initially suppressed during a normal erection is there a risk of later discharges in penile vasomotor nerves during the act? Several reports have shown that in man systemic arterial blood pressure and heart rate increase during coition or masturbation, with a peak at orgasm (Boas & Goldschmidt 1932, Klumbies & Kleinsorge 1950, Bartlett 1956, Masters & Johnson 1966, Fox & Fox 1969, Littler et al. 1974, Nemec et al. 1976). The peripheral resistance increases (Klumbies & Kleinsorge 1950, Littler et al. 1974) and during orgasm the baroreflex seems to be over ridden (Littler et al. 1974). Furthermore, the respiratory pattern changes (Klumbies & Kleinsorge 1950, Bartlett 1956, Fox & Fox 1969) and there is an increased minute volume after climax (Fox & Fox 1969) indicating that a respiratory deficit is acquired during the height of the act. These are conditions that might, according to the present results lead to increased firing in penile vasomotor nerves.

Consequently our present findings may directly point to the presumptive value of cholinergic efferent fibres in smooth muscle effectors of penile erection, because activity in such fibres might produce a peripheral restriction of the transmitter output from the dominant adrenergic vasomotor nerves (vide Klinge & Sjöstrand 1977, Klinge, Eriksson & Sjöstrand 1978). Our preceding study (Sjöstrand & Klinge 1979) indicated that at least in some rabbits the cholinergic fibres of the penis may receive their preganglionic supply preferentially via the hypogastric nerves. In this context it may be indicative that erectile responses elicited by hypogastric nerve stimulation sometimes were more resistant to the counteracting effect of carotid occlusion than those elicited by pelvic nerve stimulation. One should however be aware of the fact that intact hypogastric nerves are not necessary for penile erection in every male, neither are intact pelvic nerves (cf. Sjöstrand & Klinge 1979). Thus in rabbit hypogastric denervation does not prevent erection (Hodson 1964) and in man the corresponding operation, i.e. presacral neurectomy usually does not impair erection (Jacobson et al., 1944).

Finally it should be emphasized that although the penile adrenergic excitatory fibres are strong opponents to the vasodilator nerves it seems easier to elicit erection of a penis with intact vasomotor nerves than of a chronically decentralized one (Sjöstrand & Klinge 1979). In other words, vasodilator impulses seem to relax more effectively penile smooth muscle that is governed by normal resting activity in innervated key cells which are directed by tonic adrenergic discharges than muscle ruled by intrinsic activities and local feed-back mechanisms. There is clinical evidence supporting this statement. Thus Whitelaw & Smithwick (1951) reported that transthoracic (Th 1/Th 2–Th 11/Th 12) sympathectomy produced disturbance of erection in more than 50% of their patients. In view of the findings of e.g. Boas (1954) suggesting that

pelvic vasomotor supply in man may arise from the thoracic roots we find it likely that transthoracic sympathectomy implies decentralization and possibly also denervation of penile vasomotor neurons which would lead to an autoregulated penis that is less sensitive to low frequency discharges in the erectile fibres than is a penis governed by resting vasomotor tone (Sjöstrand & Klinge 1979)

We wish to thank Mrs Annika Rosén and Mr Einari Liukkela for excellent assistance in making the figures Mrs Kerstin Westling and Mrs Rosén for excellent and patient technical assistance Miss Eva Svensson for patient preparation of the manuscript and Mr Jan Svensson for skilled design of the electrodes. The study was supported by grants from the Magnus Bergvall's Memorial Fund and the Swedish Medical Research Council (project number B 76-04X-04753) (to N S) and by a scholarship from the Medical Research Council of the Academy of Finland (to E K)

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Intramural blood flows and flow distribution in the feline small intestine during arterial hypotension

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CASSUTO J CEDGÅRD S HAGLUND U REDFORS, S & LUNDGREN O Intramural blood flows and flow distribution in the feline small intestine during arterial hypotension. Acta Physiol Scand 1979 106 335–342 Received 18 Jan. 1979 ISSN 0001 6772. Department of Physiology and Department of Surgery II University of Göteborg, Sweden.

The vascular reactions of the parallel-coupled vascular sections of the small intestine were studied during hypotension at two different levels of intestinal arterial inflow pressure using a ^{85}Kr elimination technique. The regional hypotension was accomplished by partially occluding the superior mesenteric artery with clamp and maintained for 2 h. At the higher level (50–55 mmHg) total intestinal blood flow decreased but not to the same relative extent as blood pressure due to the autoregulatory capacity of the intestinal vascular bed The flow autoregulation was also reflected in decreased blood flow resistance. The distribution of blood to the muscularis and mucosa-submucosa layer respectively did not change significantly during or after hypotension as compared to the prehypotensive level, since the relative flow decrease was the same in the mucosa-submucosa and in the muscularis At the lower arterial pressure level (30–35 mmHg) more marked decrease of intestinal blood flow and flow resistance was observed as compared to the experiments performed at the 50–55 mmHg pressure level Moreover muscularis blood flow was relatively more decreased than blood flow in the mucosa-submucosa implying the fraction of total blood flow diverted to the muscularis was significantly decreased Despite this redistribution of blood flow histological damage was apparent only in the mucosa, particularly at the villous tips.

Key words Intestinal blood flow arterial hypotension autoregulation

The effects of a prolonged arterial hypotension on the series-coupled vascular sections (resistance exchange and capacitance vessels) in the small intestine of the cat have earlier been studied in this laboratory with a plethysmographic technique (Haglund 1973 *b* Haglund & Lundgren 1972 *b* 1973a, *b*). However the intestinal vascular bed consists also of parallel-coupled vascular circuits supplying the different wall layers of the intestine The vascular reactions in the villi and in the whole mucosa during a 5–10 min period of arterial hypotension were earlier investigated with an indicator dilution method (Lundgren & Svanvik 1973) but the intramural blood flows during prolonged arterial hypotension have not been studied The present study was performed to investigate these problems as they would be of particular interest for the pathogenesis of the intestinal mucosal lesions observed after prolonged hypotensive states both in animals and man (Chiu et al 1970 Åhren & Haglund 1973 Haglund et al 1975 1976*a b*)

METHODS

The experiments were performed on 14 cats weighing 2 5–4 kg and anesthetized with chloralose (50 mg/kg b w) after induction with ether The cats were deprived of food for 12 h but had free access to water None of the cats had any obvious signs of intestinal diseases.

Operative procedures and recordings of blood flows and pressures

A segment corresponding to about 75% of the small intestine was isolated with intact vascular supply after midline

pelvic vasomotor supply in man may arise from the thoracic roots we find it likely that transthoracic sympathectomy implies decentralization and possibly also denervation of penile vasomotor neurons which would lead to an autoregulated penis that is less sensitive to low frequency discharges in the erectile fibres than is a penis governed by resting vasomotor tone (Sjöstrand & Klinge 1979)

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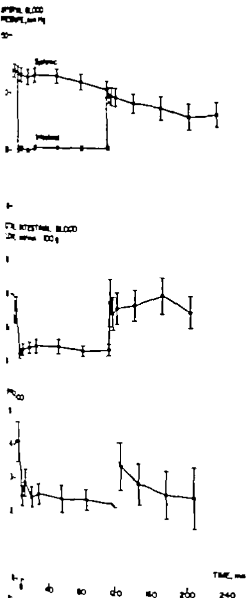


Fig 1 Arterial blood pressure, total intestinal blood flow and flow resistance before, during and after 2 h period of regional arterial hypotension at 50–55 mmHg. Bars depict S.E.

blood flow and flow resistance remained at this level throughout the period of *regional hypotension*. During the 2 h period following the arterial hypotension total intestinal blood flow was somewhat higher than prehypotensive control. Upon releasing the partial occlusion blood flow resistance initially returned to prehypotensive control

Table 1 *Blood pressure decrease (mmHg) during the period following the intestinal arterial hypotension Mean±S.E.*

	1 h	2 h
50–55 mmHg series (n=5)	28±13	44±17
30–35 mmHg series (n=5)	49±18	6.±19

but then fell continuously in the 2 h posthypotensive period reflecting a constant intestinal blood flow in the face of a slight arterial pressure reduction

During the period of regional hypotension systemic arterial blood pressure decreased from 119±6 to 103±7 mmHg, to be further reduced in the post hypotensive period (Fig. 1 and Table 1) The values given in Table 1 only include those in which a histological examination was performed. Two out of 8 animals died during the posthypotensive period in this series

In the prehypotensive control period muscularis and mucosal-submucosal blood flows amounted to 19±2 and 36±6 ml/min 100 g respectively Approximately 70% of the total intestinal blood flow was directed to the mucosal-submucosal part of the small intestinal wall Upon lowering intestinal arterial pressure to 50–55 mmHg blood flow in the muscularis and mucosa-submucosa decreased to 10±1 and 25±2 ml/min 100 g, respectively (Fig. 2) The relative flow distribution to the two layers remained however almost constant during arterial hypotension as compared to control Upon releasing the occlusion blood flow in the mucosa-submucosa and in the muscularis increased while flow distribution remained fairly constant (Fig. 2)

Histological observations The microscopic examination of the small intestine taken from cats

Table 2. *Microscopic grading of intestinal mucosa in the two experimental series of the present study*
The grading system proposed by Chiu et al. (1970) was used. Figures denote number of animals

	Grade of mucosal lesion					
	0	1	2	3	4	5
50–55 mmHg series	2	1		1		1
30–35 mmHg series			1		2	2

laparotomy The remainder of the small intestine the colon spleen, greater omentum and most of the pancreas were extirpated The right adrenal gland was ligated and the left one denervated The nerves fibres surrounding the superior mesenteric artery containing the postganglionic sympathetic vasoconstrictor fibres to the small intestine were divided

The animals were heparinized and the superior mesenteric vein, draining the small intestine and its lymph nodes was cannulated The intestinal venous blood was returned to the external jugular vein via a drop counter and a funnel By this technique the intestinal venous outflow pressure could be set to any desired level and was maintained at 10 mmHg throughout the experiments An adjustable clamp was placed around the superior mesenteric artery close to the aorta and a distal branch of this artery was cannulated for monitoring arterial inflow pressure to the intestine The left femoral artery was cannulated for systemic arterial blood pressure recording The pressures were measured by Statham transducers (P23) and recorded on a Grass polygraph A small branch of one of the mesenteric arteries supplying the intestine was cannulated with a thin polyethylene catheter to permit close i a injection of the radioactive tracer

To estimate the blood flow in the muscularis layer of the small intestine the elimination of ^{85}Kr β-radioactivity from the small intestine was followed after an intraarterial injection of the tracer β radioactivity was recorded by means of a Geiger Müller tube (Phillips No 18504 or 18550) placed at the antimesenteric border of the small intestine The G-M tube was coupled to a ratemeter (Packard Auto Gamma series 410 A) operating an ink writer Ninety per cent of uniformly distributed ^{85}Kr activity recorded by the external G-M tube emanated from the outer 0 7 mm of the intestinal wall (Thorburn et al 1966) Hence the radioactivity registered by this detector was in most experiments localized to the serosa and the muscularis. When counts exceeded 5 000 per min correction was made for coincidence loss assuming a dead time value of the G-M tube of 100 (18504) or 45 μs (18550)

Background was subtracted and the counts per minute were plotted vs time on semilogarithmic paper In most expts a monoexponential decay was recorded When a multiexponential curve appeared the monoexponential "tail" of the curve was used for determining muscularis flow (cf Kampp & Lundgren 1968) Blood flow (F) in ml per min and 100 g was calculated from equation

$$F = k \lambda \; 100 \tag{1}$$

(Kety 1951 1960) where k denotes the clearance constant and λ the blood–tissue partition coefficient k can be calculated from the equation

$$k = \frac{\ln 2}{t_{\frac{1}{2}}} \tag{2}$$

where $t_{\frac{1}{2}}$ denotes the half time of disappearance in min λ was assumed to be 1 for ^{85}Kr (Kampp & Lundgren 1968 Hultén et al. 1976*a* *b*) With this technique musculari blood flow could be followed continuously in most experiments and the i.a. injection of ^{85}Kr was repeated as count-

ing rate approached background. From direct recording of the total intestinal blood flow and the determination of the relative weights of the muscularis and the mucosa-submucosa layers in each cat mean blood flow in the mucosa-submucosa could be calculated.

A slow i v infusion of a glucose-bicarbonate solution (10 mmol $NaHCO_3$ per 100 ml 10% glucose 0.1-0 2 ml/min) was started at the induction of anesthesia and continued throughout the expt. This infusion maintains arterial pH at a normal level despite the operative trauma and the intestinal hypotension (Haglund & Lundgren 1972*a*). The tubes and the funnel draining the intestinal venous blood were primed with a dextran solution (1 low molecular weight dextran, mean M W 40 000 and 1 medium molecular weight dextran mean M W 70 000 Pharmacia, Sweden) Furthermore 2 3 ml of this solution was given to the animals every 20 min during the hypotensive period

Histological procedures

At the end of the expts the small intestine was rapidly excised weighed and a rectangular piece (about 2x4 cm) from the mid-gut portion was cut, mounted on cork and fixed in 10% neutral formalin. The blocks were prepared for routine paraffine embedment cut at about 4 μm mounted and stained with v Gieson. The mucosal lesions were graded as described earlier (Chiu et al 1970, Ahren & Haglund 1973) into 6 grades where grade 0 means normal mucosa and grades 1 to 5 denote increasing damage to the surface epithelium Pathognomonic for the grade 1 lesion is the development of a subepithelial space at the tip of the villus. This space is more extensive in grade 2 where also epithelial lifting is seen. In grade 3 a massive epithelial lifting occurs also along the sides of the villi and in grade 4 the villus is denuded Grade 5 is characterized by disintegration of the mucosal lamina propria, hemorrhage and ulceration but it is pathognomonic that deeper wall layers are microscopically normal

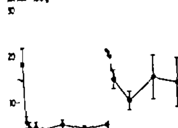
Experimental procedures

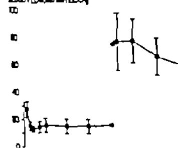
After the operative procedures had been completed a period of 30–40 min was allowed to obtain steady control measurements of systemic blood pressure and of muscularis and total intestinal blood flows. A h regional intestinal hypotension was then produced by lowering the intestinal arterial inflow pressure to 30–35 (n 6) or 50-55 mmHg ($n = 8$) by means of mesenteric artery obstruction. The experiments were continued for another h after the h period of regional intestinal hypotension

RESULTS

Prolonged hypotension at 50–55 mmHg

Hemodynamic observations Lowering the intestinal arterial inflow pressure from about 120 to 50-55 mmHg reduced within 10 min total intestinal blood flow from 31±4 to 19±2 ml/min 100 g (mean value ± S E $n = 8$) and regional intestinal blood flow resistance from 4 1±0 6 to 2.5±0 3 PRU_{100} (Fig 1).





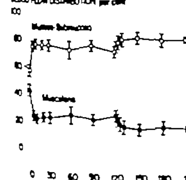


Fig. 4. Blood flow and flow distribution in the muscularis and the mucosa-submucosa of the small intestine before, during and after a 2 h period of arterial hypotension (30-35 mmHg). Note that blood flows are expressed per 100 g of the respective tissue. Bars denote S.E. n=6.

hypotensive period. Upon release of the partial arterial occlusion, intestinal blood flow increased well above prehypotensive control to about 50 ml/min 100 g, and remained elevated throughout the rest of the expts. Flow resistance exhibited a peak upon the release of the partial occlusion and returned within 20–40 min to values similar to those recorded during the hypotensive period.

Systemic arterial blood pressure fell from 130±8 to 115±4 mmHg during the period of regional intestinal hypotension. A further marked reduction was recorded in the posthypotensive period (Fig. 3) and 2 of the 6 animals died during the 2 h period following intestinal hypotension. The blood pressure changes reported in Table 1 are those observed in the 5 animals in which histological examinations were performed.

As shown in Fig. 4 the mucosal-submucosal blood flow was reduced to about half of control 10 min after lowering the intestinal arterial blood pressure and remained fairly constant throughout the hypotension. The muscularis blood flow on the other hand, was reduced to about 30% of control during the hypotensive period. These flow values indicate that a larger fraction of intestinal blood flow was diverted to the mucosa-submucosa during low pressure as compared to control (see Fig. 4). During the posthypotensive period an initial transient hyperemia was apparent in the muscularis. The blood flow rate in the mucosa-submucosa increased to a level 2–3 times above control and remained there for the rest of the expt. More than 80% of total intestinal blood flow was then diverted to the mucosa-submucosa.

Histological observations. The microscopic examination of the intestinal segments revealed various degrees of mucosal damage (Table 2). Some cats exhibited total destruction of the villi.

DISCUSSION

In the present study the reactions of the parallel-coupled vascular beds of the small intestine (Folkow 1967) were studied when exposed to 2 different levels of regional hypotension. At the higher level of arterial pressure (50–55 mmHg) total intestinal blood flow decreased though not to the same extent as pressure thanks to the autoregulatory capacity of the intestinal resistance vessels, as also reflected by a decreased resistance to blood flow. The flow distribution to the muscularis and mucosa-submucosa layer remained largely unchanged during or after hypotension as compared to control. In 3 of 5 animals no mucosal lesions were observed. The two other animals exhibited mucosal lesions and they died during the course of the expts.

At the lower level of arterial pressure (30–35 mmHg) more marked decreases of both flow and resistance were observed as compared to the 50–55 mmHg arterial pressure level. Moreover muscularis blood flow was decreased relatively more

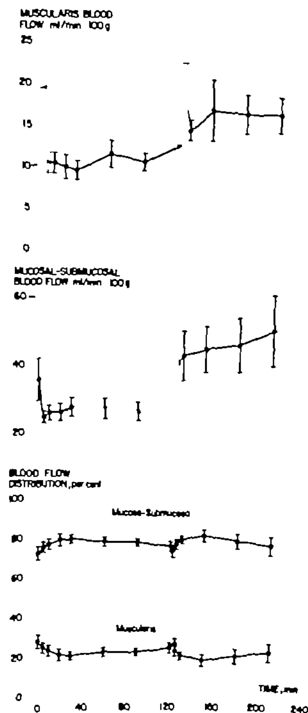


Fig 2 Blood flow and flow distribution in the muscularis and in the mucosal-submucosal layers of the small intestine before during and after a 2 h period of regional arterial hypotension at 50–55 mmHg Note that blood flows are expressed per 100 g of the respective tissue Bars denote S E n=8.

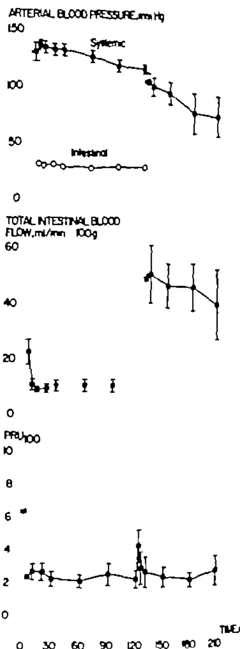


Fig 3 Arterial blood pressure total intestinal blood and flow resistance before during and after a 2 h peri regional arterial hypotension at 30–35 mmHg Bars d S E n=6

subjected to hypotension at the 50–55 mmHg level revealed a normal mucosa in 3 of 5 animals (Table 2) In the remaining two animals marked ulcerations were observed These two animals died during the course of the expts

Prolonged hypotension at 30–35 mmHg

Hemodynamic observations Lowering of the in tinal arterial inflow pressure to 30–35 mmHg duced within minutes total intestinal blood f from 22±4 to 9±1 ml/min 100 g (n=6) (Fig Concomitantly intestinal blood flow resistance from 6 4±1 2 to 2 2±0 5 PRU$_{100}$ revealing marked relaxation of the vascular smooth musc Total intestinal blood flow and resistance remai fairly unchanged during the remainder of the

on despite an almost unchanged villous blood r can be satisfactorily explained only by the ence of a villous countercurrent exchange of ies between the central arterial vessel and subepithelial network of villous capillaries 1967). One solute which should be par ly easily "trapped" in the exchanger is gen, since P_{O_2} is higher in the supplying arterial el than in the subepithelial capillaries. A rtcircuiting of oxygen in the villous countercur t exchange has been described during normoten se conditions (Kampp et al. 1968). In hypotension e volume of blood supply to the villi seems to be gely unchanged while the linear flow velocity is ch reduced (Lundgren & Svanvik 1973). This, in n, is one factor that enhances the "oxygen trap g efficiency of the exchanger. This shortcircuit g oxygen at the villous base renders the villous s severely hypoxic, despite a fairly normal blood w (for a more thorough discussion of this chanism, see Lundgren & Haglund 1978*b* and idal et al. 1978).

Intestinal mucosal damage of the characteristic type found in this study has been reported in other hypotensive studies (Bounous 1967, Chiu et al. 1970, Åhrén & Haglund 1973, Lundgren & Haglund 1976*a*, *b*). It might be argued that the intestinal lesions were not present immediately after the intestinal hypotensive period but developed during the 2 h control period following the hypotension. However, in all earlier reports from this laboratory intestinal biopsies have been taken as early as 1 h after the shock period and showed similar ulcerations as reported here (Åhrén & Haglund 1973, Haglund et al. 1976*a*). Furthermore, in experiments primarily devoted to collect intestinal venous blood for biochemical analysis, intestinal tissue samples were taken within 30 min after the hypotensive period and these segments exhibited similar lesions (Lundgren & Haglund 1978).

Based on rather extensive experimental studies it has been suggested that the mucosal damage aggravates the shock state (Haglund & Lundgren 1973*b*, Haglund et al. 1976*b*) presumably via a release of cardiotoxic material (Lundgren et al. 1976, Lundgren & Haglund 1978*b*, Haglund et al. 1978). This conclusion was further strengthened by the present observation that the fall in systemic arterial pressure after the 2 h period of intestinal hypotension was considerably less pronounced in the expts. performed at 50–55 mmHg, where the mucosal appearance remained largely normal, than in the 30–35 mmHg series, where pronounced mucosal ulcerations ensued.

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Table 3 *Blood flow and flow distribution in the feline small intestine during control conditions and during arterial hypotension at 50–55 mmHg and 30–35 mmHg*

The values represent average values calculated from the results of the present study and those reported by Lundgren & Svanvik (1973)

	Control	Arterial blood pressure 50–55 mmHg	Arterial blood pressure 30–35 mmHg
Blood flow (ml/min×100 g)			
Total	30	20	10
Villi	40	40	30
Crypts	50	20	6
Submucosa	17	10	4
Muscularis	17	10	4
Blood flow distribution (% of total flow)			
Villi	30	45	65
Crypts	40	25	13
Submucosa	5	5	4
Muscularis	25	25	18

than the mucosal-submucosal blood flow implying a more pronounced compensatory vasodilatation in the mucosa and submucosa layers Despite this redistribution of blood flow histological damage was seen only in the villous parts of the mucosa (Table 2)

In an earlier study by Haglund & Lundgren (1972*a*) the reactions of the series-coupled vascular sections were studied during experimental conditions closely similar to those of the present study The changes in total intestinal blood flow and flow resistance recorded in this study corresponded well with the results reported by Haglund & Lundgren (1972*a*) This also holds true for the decrease in the systemic arterial blood pressure observed in the period following regional intestinal hypotension (Table 1)

The blood flow and flow distribution in the intestinal wall has earlier been studied during comparatively short periods of arterial hypotension (Lundgren & Svanvik 1973) using an indicator dilution technique allowing the villous or the mucosal blood flow to be followed together with the total venous outflow These authors found that villous blood flow remained fairly constant in the face of a considerably reduced total intestinal blood flow when the perfusion pressure was reduced from 100 to about 25 mmHg Similar results were also re ported by Bond & Levitt (1976) during hemorrhag hypotension in dogs studied with a microspher technique In agreement with those findings it w observed in the present study that blood flow in th mucosa-submucosa was reduced upon hypotensi but to a less extent than total intestinal blood flo and considerably less than muscularis blood flow.

The results of the present study suggest that th hemodynamic situation present in the small inte tine 10 min after lowering perfusion pressure maintained throughout the 2 h hypotensive peri (Figs 2 and 4) Combining the results reported l Lundgren & Svanvik (1973) on villous blood flo recorded within 10 min hypotension and the pr ent results it is possible to calculate blood fl distribution quantitatively in the feline intesti wall during arterial hypotension This has been c ried out in Table 3 It was assumed that vill blood remained largely the same (50–55 mmHg) only slightly reduced (30–35 mmHg) as compared control (cf Lundgren & Svanvik 1973) Furth more the submucosal blood flow was presu to be the same as that of the muscularis and relative weights of the different tissue comp ments was assumed to be the following' vill crypts 23 submucosa 10 and muscularis 45

Two observations stand out with regard to Ta 3 First a slight redistribution from the muscul towards the mucosa occurs although it is not prominent as the one illustrated in Fig. 4 T reason for this discrepancy is that total intesti blood flow in the control situation in Table 3 higher than in the expts. illustrated in Fig. 4 T implies in turn that the fraction of blood f diverted to the muscularis is higher in the Fig. t in the Table Second the fraction of total intest blood flow distributed to the villi is higher the lo the arterial blood pressure The villous fractio creases from 30% during control to 65% at an terial pressure of 30–35 mmHg (cf Bond & Le 1976)

Evidently villous blood flow is fairly well m tained in the face of even pronounced decrease arterial pressure Nevertheless tissue lesions v only seen in the villi and particularly at their ti agreement with earlier reports (Åhrén & Hag 1973 Haglund et al 1976*a b*) Moreover t villous lesions have been demonstrated to primarily caused by tissue hypoxia (Haglund e 1976*a*) The apparent paradox of hypoxic vil

Acta Physiol Scand 1979 106: 343–350

Studies on ocular blood flow and retinal capillary permeability to sodium in pigs

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TÖRNQUIST, P., ALM, A. & BILL, A. Studies on ocular blood flow and retinal capillary permeability to sodium in pigs. Acta Physiol Scand 1979. 106: 343–350. Received 22 Jan. 1979. ISSN 0001-6772. Institute of Physiology and Medical Biophysics and Department of Ophthalmology, University Hospital, University of Uppsala, Sweden.

A surgical technique was developed in pigs that permits access to the retinal venous plexus surrounding the optic nerve. The effect of surgery on ocular blood flow and capillary permeability was evaluated. Blood flow, determined by the labelled microsphere technique, did not differ significantly between operated and control eyes. Increased intraocular pressure in the operated eye reduced blood flow through the choroid and the anterior uvea in proportion to the reduction in perfusion pressure, while in the retina a smaller reduction in blood flow occurred indicating that autoregulatory mechanisms are involved in the control of retinal blood flow. The capillary permeability to sodium was studied by the single injection technique, using albumin as a reference substance. The fractional initial extractions from the choroidal and the retinal vessels were 0.77 and −0.001, respectively. The absence of a sodium extraction from the retinal vessel indicates that this part of the blood-retinal barrier was intact and that the blood drained by the retinal plexus is not mixed with blood from other sources.

Key words: Ocular blood flow, retinal capillary permeability, labelled microspheres, single injection technique

The metabolic needs of the retina are supplied from two sources, the retinal capillaries and the choriocapillaris. The retinal capillaries have a continuous endothelial lining with tight junctions, while the choroidal capillaries are fenestrated (Bernstein & Hollenberg 1965). The endothelial cells of the retinal capillaries constitute a diffusion barrier to water-soluble substances such as sodium fluorescein (Grayson & Laties 1971) and microperoxidase (Smith & Rudt 1975). This barrier has an epithelial analogue in the RPE, which prevents diffusion of water-soluble substances into the retina from the highly permeable choriocapillaris (Shiose 1970). These two barriers constitute the BRB.

The primary function of the circulation is to supply nutrients and to remove waste products. The choroidal contribution of glucose to the retinal metabolism has previously been studied in cats (Törnquist 1979). Arterial and choroidal venous blood was collected and the a-v difference for glucose was determined. Unsuccessful efforts were also made to obtain retinal venous blood. Simultaneous collection of retinal and choroidal blood would provide quantitative information about the respective roles of the retinal and choroidal vessels in the nutrition of the retina.

The pig has a retinal circulation that bears a close resemblance to that of man. Preliminary studies on enucleated pig eyes showed that the retinal veins drain into a circular venous plexus around the optic nerve close to the globe, and that it was possible to cannulate this plexus. Retrograde injections of a silicone rubber (Microfil, Canton Biomedical Products, Inc., Colo) into this plexus filled the retinal veins, but revealed no anastomoses to the choroid. Uveal blood can also be obtained by cannulating one of the vortex veins. These vessels drain blood

Abbreviations: BBB = blood-brain barrier, BRB = blood-retinal barrier, RPE = retinal pigment epithelium, IOP = intraocular pressure, MAP = mean arterial blood pressure and PP = perfusion pressure (PP = MAP − IOP).

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Table 1. Blood flow through the various tissues of the control and the experimental eye at different PP

(Blood flow values are in mg/min per whole tissue, except for the optic nerve, where it is calculated as g/min per 100 g; mean ±S.E. (n = number of animals))

Tissue	Sphere injection I (n = 6) Control eye PP 138 (range 120–165)	Sphere injection I (n = 6) Experimental eye PP 127 (range 120–135)	Sphere injection II (n = 5) Control eye PP 132 (range 105–150)	Sphere injection II (n = 5) Experimental eye PP 85 (range 75–100)	Sphere injection III (n = 6) Control eye PP 120 (range 75–145)	Sphere injection III (n = 6) Experimental eye PP 50 (range 45–60)
Retina	61 ±8	64 ±7	76 ±4	61 ±5	65 ±3	23 ±5
Iris	112 ±42	166 ±21	181 ±6	87 ±11	193 ±32	30 ±8
Ciliary body	313 ±56	380 ±30	420 ±69	213 ±41	428 ±80	82 ±22
Choroid	1 646 ±202	1 732 ±134	1 610 ±102	930 ±122	1 617 ±175	342 ±84
Optic nerve retrobulbar part	14 ±2	18 ±2	15 ±3	20 ±3	16 ±2	14 ±1

choroid and the retina as previously described (Alm & Bill 1973). When preparing these preparations care was taken to ensure that the part of the choroid and the retina corresponding to the cannulated vortex vein could be identified.

The flat mount preparations were divided to permit determination of blood flow per area. Thus from each flat mount four squares, each of 20–30 mm², were taken: two from the periphery and two from the central parts. The radioactivity of each square and of the remaining parts of the flat mount preparation was determined. In eyes where the vortex vein had been cannulated, one peripheral and one central square were taken from the quadrant drained by the vein and from the opposite quadrant.

The reference blood samples were weighed and the radioactivity of all samples was determined by three-channel gammaspectrometry. Blood flow for the various tissues was calculated by dividing the activity of the tissue sample by the activity per mg blood flow per min calculated for the reference flow.

Permeability studies

The permeability of retinal and choroidal capillaries to albumin was studied in 11 pigs by the single injection technique (Crone 1963, Törnquist 1977, 1979). After an injection of 2 tracers (one test and one reference substance) simultaneously obtained venous outflow curves (concentration vs. time) provide information of transcapillary exchange of the test substance. This is based on the assumption that there is no separation of the tracers in the intravascular space and that the reference substance is restricted to the intravascular volume during the passage through the tissue. In studies on the choroid in cats albumin was shown to fulfil the criteria of a reference tracer (Törnquist 1977), and was chosen as the reference in the present experiments.

The albumin was prepared from rabbit plasma with conventional techniques. The purity was checked with disc electrophoresis. It was labelled with ¹²⁵I and purified from free iodide by gel chromatography on Sephadex G-25 coarse (Pharmacia, Sweden) on the day of the experiment. ²²NaCl (NEN USA) was used as test substance. The substances were dissolved in plasma obtained from the experimental animal. A volume of about 0.5–1.0 ml was injected into an exposed carotid artery over less than 2 s. Venous blood samples were collected from the retinal vein on a strip of filter paper and in 3 expts. simultaneously from a vortex vein. The blood was sampled for 15–20 s and at a rate of about one sample per s. The blood was analysed for radioactivity. The fractional extraction $E_{(t)}$ is given by the formula

$$E_{(t)} = (C_{ref} - C_{test}) / C_{ref}$$

Here C indicates the actual concentration of the tracers expressed as a fraction of the concentration in the injectate. For presentation of the results three different extraction values were defined. E_{in} is the initial extraction calculated from the first sample where the activity of the reference substance was at least three times that in the samples collected before the injection. The counting time was chosen to obtain more than 1 000 counts after background subtraction. $E_{max\,alb}$ is the fractional extraction at the highest albumin concentration. E_{net} is the difference in areas between the outflow curves divided by the area of the albumin curve. The areas were calculated from the appearance of the tracers to the time when albumin concentration had fallen to about 20 % of the peak value. This limit was chosen to avoid interference of recirculation.

In one animal an intracarotid injection of hypertonic NaCl (3 ml of 10 osmol solution injected over 15 s) was made a few min before a single injection expt. to determine if the single injection technique could reveal an osmotic opening of the BRB.

RESULTS

Ocular blood flow determinations

In one pig ocular blood flow was determined twice, and in 5 pigs three times at different IOP with an interval between flow measurements of at least 30 min. Since both MAP and IOP were recorded PP

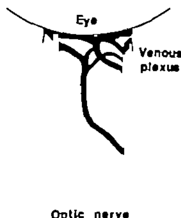


Fig. 1 The anatomy of the venous plexus on the optic nerve See text

mainly from the choroid and to some extent from the anterior uvea that is the iris and the ciliary body

A prerequisite for meaningful determinations of blood flow and capillary permeability is that surgery itself does not affect these factors The purpose of the present study can be summarized in the following aims

1 To establish whether the surgical procedures necessary to obtain retinal and uveal blood affected the blood flow

2 To obtain values for normal blood flow through the various parts of the pig s eye

3 To determine the effect of increments in IOP on blood flow in eyes with one vortex vein and the retinal venous plexus cannulated

4 To determine the permeability of the retinal capillaries to sodium

METHODS

General procedures

Young pigs of both sexes weighing between 19 and 27 kg were used Anesthesia was induced and maintained by i v injections of pentobarbital sodium (Mebumal Vet ® ACO Sweden) Atropine (0.5 mg) was given i v to reduce bronchial secretion The body temperature was kept normal with a heating pad Two arteries and one vein were cannulated with polyethylene catheters One femoral artery was connected to a pressure transducer for measurements of the MAP One brachial artery was used for blood sampling, and a continuous saline infusion (20 drops per min) was given into one brachial vein All animals were tracheotomized and if needed artificially ventilated with a respirator adjusted to give normal values for arterial blood gases. Arterial P_{O_2} P_{CO_2} and pH were determined with an automatic analyser (ABL 1 Radiometer Denmark)

Preparation of the eye

In each pig the surgical procedure was performed in one eye only the other eye served as control. The superficial muscles the cranial part of the temporal muscle and the lateral wall of the orbit were removed thus exposing the lateral part of the eye An incision was made in the conjunctiva for 90° along the lateral limbus The incision was extended in meridional directions. The conjunctiva and the underlying extraocular muscles, including part of the retractor bulbi were then removed In order to expose the retinal venous plexus some fat tissue had to be removed The anatomy of the plexus varied but a constant finding was a venous ring around the optic nerve just outside the globe and a number of draining vessels running along the optic nerve (Fig. 1)

A polyethylene catheter was introduced into the venous plexus through a small incision The tip of the catheter was advanced close to the globe The catheter was made as short and wide as possible. As a rule about 5 mm of slightly tapered polyethylene tubing, external diameter about 1 mm (Portex Kent UK) was used To prevent backflow from the draining veins some of them were coagulated To facilitate the cannulation two or three stabilizing sutures were placed in the conjunctiva near the limbus Only light traction was used to rotate the globe in order to avoid compromising the circulation Finally the temporal vortex vein was cannulated Before the ocular veins were cannulated 50000 I U of heparin was given i v In some experiments indomethacin 60–100 mg dissolved in a phosphate buffer was given to reduce platelet adhesiveness.

The anterior chamber was cannulated with two steel cannulas one connected to a pressure transducer for monitoring IOP and the other connected to a reservoir containing mock aqueous humor (Bárány 1964) for artificial regulation of the IOP

Ocular blood flow measurements

In 6 pigs ocular blood flow was determined with the labelled microsphere method Microspheres with a diameter of 15 μm labelled with ^{46}Sc ^{85}Sr or ^{141}Ce (3 M Company St Paul Minn.) were used $4–8 \times 10^6$ spheres, specific activity 10 mCi/g were injected into the left ventricle of the heart exposed through a thoracotomy Since blood flow determinations were made at different levels of IOP in the same animal 2 or 3 determinations were made with differently labelled spheres Sphere injection I was made with the spontaneous IOP in both eyes From the start of the injection and for the following 60 s blood was sampled from the cannulated brachial artery to serve as a reference blood flow IOP was then increased by 30–50 cmH_2O in the experimental eye and about 30 min later sphere injection II was made in the same way In 5 of 6 pigs IOP in the experimental eye was then increased by another 30–50 cmH_2O and 30 min later sphere injection III was made The control eye was kept at spontaneous IOP during all three blood flow determinations At the end of the last blood flow determination the animals were killed by an intracardiac KCl injection Both eyes were enucleated and dissected Iris ciliary body and the retrobulbar part of the optic nerve 6–10 mm were taken as separate weighed samples while flat mount preparations were made of the

[illegible] 1. Blood flow through the various tissues of the control and the experimental eye at different PP

[illegible] values are in mg/min per whole tissue, except for the optic nerve where it is calculated as g/min per 100 g [illegible] Mean ±S.E. (n = number of animals)

	Sphere injection I (n = 6)		Sphere injection II (n = 5)		Sphere injection III (n = 6)	
	Control eye PP 138 (range 120–163)	Experimental eye PP 127 (range 120–135	Control eye PP 132 (range 105–150	Experimental eye PP 85 (range 75–100	Control eye PP 120 (range 75–145	Experimental eye PP 50 (range 45–60
[illegible]	61 ±8	64 ±7	76 ±4	61 ±3	65 ±3	23 ±5
[illegible]	112 ±42	166 ±21	181 ±6	87 ±11	193 ±32	30 ±8
Ciliary body	313 ±36	380 ±30	420 ±69	213 ±41	428 ±80	82 ±22
[illegible]oid	1 646 ±202	1 732 ±134	1 610 ±102	930 ±122	1 617 ±175	342 ±84
Optic nerve retro-[illegible] part	14 ±[illegible]	18 ±2	15 ±3	20 ±3	16 ±2	14 ±1

[illegible] and the retina as previously described (Alm & Bill [illegible]). When preparing these preparations care was taken [illegible] that the part of the choroid and the retina cor[illegible]ponding to the cannulated vortex vein could be [illegible].

The flat mount preparations were divided to permit [illegible] of blood flow per area. Thus from each [illegible] four squares, as a rule 20–80 mm² were taken, tw[illegible] the periphery and two from the central parts. The [illegible]activity of each square and of the remaining parts of [illegible] flat mount preparation was determined. In eyes where [illegible] vortex vein had been cannulated, one peripheral and [illegible] central square were taken from the quadrant drained [illegible] the vein and from the opposite quadrant.

The reference blood samples were weighed and the [illegible]activity of all samples was determined by three [illegible] gamma spectrometry. Blood flow for the various [illegible] was calculated by dividing the activity of the tissue [illegible] by the activity per mg blood flow per min calcu[illegible] for the reference flow.

[illegible] studies

The permeability of retinal and choroidal capillaries to [illegible] was studied in 11 pigs by the single injection [illegible] (Crone 1963, Törnquist 1977, 1979). After an [illegible] of 2 tracers (one test and one reference [illegible]) simultaneously obtained venous outflow [illegible] (concentration vs. time) provide information of [illegible] exchange of the test substance. This is [illegible] on the assumption that there is no separation of the [illegible] in the intravascular space and that the reference [illegible] restricted to the intravascular volume during [illegible] passage through the tissue. In studies on the choroid in [illegible] albumin was shown to fulfil the criteria of [illegible] reference [illegible] (Törnquist 1977), and was chosen as the reference [illegible] the present experiments.

The albumin was prepared from rabbit plasma with [illegible] technique. The purity was checked with disc [illegible]phoresis. It was labelled with ¹²⁵I and purified from [illegible] iodide by gel chromatography on Sephadex G-25 [illegible] (Pharmacia, Sweden) on the day of the experiment. [illegible]Cl (NEN,USA) was used as a test substance. The substances were dissolved in plasma obtained from the experimental animal. A volume of about 0.5–1.0 ml was injected into an exposed carotid artery over less than 2 s. Venous blood samples were collected from the retinal vein on a strip of filter paper and in 3 expts. simultaneously from a vortex vein. The blood was sampled for 15–20 s and at [illegible] rate of about one sample per s. The blood was analysed for radioactivity. The fractional extraction E, is given by the formula:

$$E_{[illegible]} = (C_{ref} - C_{test})/C_{ref}$$

Here C indicates the actual concentration of the tracers expressed as [illegible] fraction of the concentration in the injectate. For presentation of the results three different extraction values were defined. E_{in} is the initial extraction calculated from the first sample where the activity of the reference substance was at least three times that in the samples collected before the injection. The counting time was chosen to obtain more than 1 000 counts after background subtraction. E_{max} is the fractional extraction at the highest albumin concentration. E_{net} is the difference in areas between the outflow curves divided by the area of the albumin curve. The areas were calculated from the appearance of the tracers to the time when albumin concentration had fallen to about 20% of the peak value. This limit was chosen to avoid interference of recirculation.

In one animal an intracarotid injection of hypertonic NaCl (3 ml of [illegible] 10 osmol solution injected over 15 [illegible]) was made [illegible] few min before [illegible] single injection expt. to determine if the single injection technique could reveal an osmotic opening of the BRB.

RESULTS

Ocular blood flow determinations

I[illegible] one pig ocular blood flow was determined twice, and in 5 pigs three times at different IOP with an interval between flow measurements of at least 30 min. Since both MAP and IOP were recorded PP

Table 2 *Blood flow through central and peripheral regions of the retina and the choroid of the experimen tal eye determined for the quadrant corresponding to the cannulated vortex vein and the opposite quadra*
The presented values were obtained at spontaneous IOP and are given as mg/min/mm³ Mean ±S.E. (n 6)

	Retina		Choroid	
Region	Cannulated	Not cannulated	Cannulated	Not cannulated
Central	0 10±0 01	0 11±0 02	3 7±0..	3 2±0 4
Peripheral	0 10±0 03	0 09±0 01	3 7±0 8	3.5±0 8

could be calculated Table 1 presents the results of these blood flow measurements with the mean actual PP—and the range—presented for each sphere injection Although MAP tended to fall slightly during the experiments the PP for the control eyes were similar during the three blood flow determinations No significant difference in blood flow between the two eyes was observed at spontaneous IOP (sphere injection I) A moderate reduction in PP in the experimental eye (sphere injection II) caused a significant decrease in blood flow compared to the control eye ($P<0$ 025 paired data) for all tissues except the optic nerve A further reduction in PP (sphere injection III) reduced blood flow still further

Table 2 presents the results of the determinations of regional blood flow for retina and choroid in the experimental eye The results obtained in the con trol eye were similar There was no significant dif ference between central and peripheral region and no significant difference between blood flow per area through the quadrant drained by the cann lated vortex vein compared to the oppo quadrant for either retina or choroid

Fractional sodium extraction in the retinal circulation

The apparent tissue extraction of sodium was ve low in the majority of experiments (see Table 3 k column) In these animals the average $E_{(i)}$=−0.00 $E_{(peak)}$=0 03 and $E_{(area)}$=0 003 These results ind cate that the tracers passed through the blood ve sels without any significant loss as can be seen f the venous outflow curves for a representativ expt (77 131 Fig 2 A) The curves are quite coi cident and the $E_{(i)}$-curve is within +0.09 and −0.1 However in 2 expts 77 188 and 77 234 the $E_{(i)}$ was considerably higher although the $E_{(area)}$ was the same magnitude as in the first group (Table 3 Fig 2 B illustrates the outflow curve of 77-23 which closely resembles that of Fig 2 A Note th pattern of the $E_{(i)}$-curve with an initial and fin

Table 3 *The fractional extractions of sodium*

For explanation of the individual experiments see text
E_i = the initial extraction $E_{(peak)}$ = the fractional extraction at the highest albumin concentration $E_{(area)}$ the ar extraction as the difference in areas between the outflow curves divided by the area of the albumin curve The areas we calculated from the appearance of the tracers until the time when the albumin concentration had fallen to about 20% c the peak value

Retina				Choroid				Retina			
Expt	$E_{(i)}$	$E_{(peak)}$	$E_{(area)}$	Expt.	E	$E_{(peak)}$	$E_{(area)}$	Expt.	$E_{(i)}$	$E_{(peak)}$	$E_{(area)}$
77 111	0 06	−0 01	0 03	77 138-2	0 79	0 19	0.27	77 188	0 31	0 16	0 07
77 131	0 09	0 00	−0 03	77 216-	0 77	0 32	0 23	77 .34	0 40	−0 13	0.01
77 138-1	−0 14	0 04	0 04	77 219-	0 75	0 44	0 18				
77 181	−0 02	0 07	−0 01					77 138-3	0 39	0 38	0 3
77 184	−0 06										
77 216-1	−0 01	0 03	0 01								
77 219-1	−0 03	0 12	0 02								
77 224	0 10	−0 04	−0 04								
Mean	−0 001	0 03	0 003								

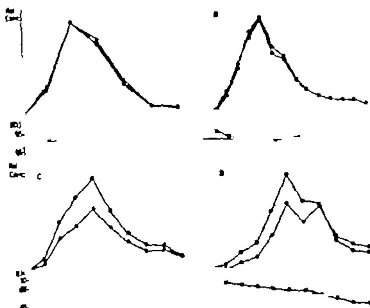


Fig. 2. The venous outflow curves and the corresponding E_{ref}-curves from four experiments. See text. (A) From the retinal venous plexus. A representative curve. (B) From the retinal venous plexus. Taylor diffusion. (C) From the retinal venous plexus. After hypertonic NaCl-injection. (D) From the vortex vein. A representative curve. ○ ^{125}I-albumin, ● ^{24}Na, □, ^{22}Na.

positive extraction and an intermediary negative extraction. This pattern was also seen in 77 188.

After an intracarotid injection of hypertonic NaCl there was a clear separation of the two tracers (Fig. 2C). The initial sodium extraction was 0.39 (77 138-3, Table 3) and the value remained positive during the entire time of observation. The extraction values can be compared to 77 138-1 in Table 3 which shows the values obtained before the hypertonic injection in the same animal. A marked increase was noted for all values. It was noticed in this experiment that blood flow from the retinal vein gradually ceased after the hypertonic injection.

Fractional sodium extraction in the choroidal circulation

The same pattern was seen in all three experiments—a high initial extraction of about 0.77, a negative slope of the E_{ref}-curve and a final negative extraction value. The accumulated loss prior to recirculation was about 3% of the sodium during the expts. (E_{mean} 0.23). Individual values are presented in Table 3 and a representative expt. in Fig. 2D. Note the similarity to expt. Fig. 2C.

DISCUSSION

Blood flow through control eyes

In pigs of similar weights as in the present study Malik et al. (1976) using 15 μm spheres found blood flow values for the retina, anterior uvea and choroid of 150, 1300 and 16500 ml per 100 g d.w. per min respectively. The mean wet weight of the anterior uvea in the present study was 103 mg. No weights were obtained for choroid or retina, but previous studies on pigs of similar weights and ages as used in the present study resulted in mean wet weights of 35 and 135 mg respectively (unpublished observation). Using these weights and assuming a dry weight/wet weight ratio of 0.20 the results of the present study, expressed in the same unit, corresponds to about 225, 2000 and 23500 respectively for retina, anterior uvea and choroid in the control eyes.

In monkeys blood flow through the central parts of the retina and the choroid, determined with 15 μm spheres, is 5–10 times larger per unit area than in the periphery (Alm & Bill 1973). No such difference was found in the pigs of the present study, or

in a previous study on cats using 15 μm spheres (Alm & Bill 1972) Neither the present study nor that on cats exclude moderate regional differences as has been suggested for cats in studies using high-speed cine fluorescein angiography (Laatikainen 1976) However the studies with microspheres show a marked difference between monkeys and the two other species

Blood flow was determined three times in the control eyes without any changes in the experimental situation between flow measurements except a slight fall in MAP The interval between flow determinations was at least 30 min to give the experimental eye ample time to adjust to the change in IOP Ideally the spheres should become definitely trapped within the capillary bed but there is a possibility that with time some trapped spheres may escape through the capillary bed into the systemic circulation If this is true one should expect flow values obtained during sphere injection I and possibly II to be lower than those obtained during sphere injection III No such differences were observed for choroid retina or optic nerve of the control eye (Table 1) Blood flow values calculated from sphere injection I for iris and ciliary body are lower than those calculated from II and III The differences are not statistically significant but it is an interesting observation since the anterior uvea seems to have the largest tendency to allow spheres to pass through the capillary bed although the capillaries of the choroid are generally assumed to be much wider (Hogan et al 1970) Thus in rabbits 50% of 8–10 μm spheres pass through the anterior uvea while they become more or less completely trapped within the retina and the choroid (Alm Törnquist & Stjernschantz 1977) and the same is true for pigs (Törnquist unpublished observation)

Blood flow through the experimental eye at normal IOP

The spontaneous IOP in the experimental eye was in general 10–15 cmH_2O higher than in the control eye presumably due to increased venous pressure caused by the extensive surgery However this caused no appreciable effect on blood flow (Table 1 and 2)

Blood flow through the experimental eye at increased IOP

In most organs moderate reductions in perfusion pressure do not result in a corresponding reduction in blood flow since a compensatory vasodilatation takes place that is blood flow is autoregulated Increased IOP as well as reduced MAP result in reduced PP for the eye Previous studies with the labelled microsphere technique on cats (Alm & Bill 1972) monkeys (Alm & Bill 1973) and rabbits (Bill 1974) have all shown autoregulation of retinal blood flow No similar study on pigs is known to us, but studies with fluorescein angiography (ffytche et al 1974) have indicated autoregulation of retinal blood flow In the present study a reduction of PP by 33 (sphere injection II) reduced blood flow through the retina of the experimental eye by 19±4% (M±S E $P<0.01$) if compared to blood flow through the control eye at sphere injection II and by 5±8% if compared to blood flow through the experimental eye at spontaneous IOP Both these comparisons seem valid and although they differ numerically both indicate some autoregulation of retinal blood flow in the present study The results, however, suggest a less efficient autoregulation than that found in the studies on cats monkeys and rabbits The discrepancy may be due to chance variation in the small material of the present study but it is also possible that the surgical trauma may have interfered with the autoregulation Such effects have been shown in the brain (Fog 1968) Increments in eye pressure did not appreciably affect the blood flow in the optic nerve behind the eye This result is in good agreement with previous observations in the monkeys (Alm & Bill 1973)

No tendency to autoregulation of blood flow was observed for either choroid or anterior uvea in the present study The lack of autoregulation in the choroid agrees with the previous microsphere studies in other species mentioned above For the anterior uvea autoregulation of blood flow has been observed in cats and monkeys but not in rabbits

Sodium permeability

Choroid The experiments on the permeability of the choriocapillaris are in agreement with previous studies on cats (Törnquist 1979) The high initial extraction is a consequence of the high permeability of the choriocapillaris The slope of the E_{in}-curve is an expression of rapid back diffusion of sodium from the tissue to the blood indicating a small extravascular volume of distribution The accumulated loss of 23% was calculated as the relative difference in area between the outflow curves up to the time when the concentration of the albumin had

falls to 20% of the peak value Later parts of the curve might be influenced by recirculation and were considered unreliable In the cat this problem was controlled and a greater part of the outflow curve could be used. This explains the finding of an accumulated loss of only 3% in the study on cats (for details see Törnquist 1979)

Retina A separation of the outflow curves when albumin is compared to sodium could be interpreted as an extravascular loss of sodium. In 2 expts (77 188 and 77-234) we found an initial fractional extraction, E_{max} of 0.31 and 0.40 indicating a transcapillary movement of sodium. However since in the majority of the expts. we found negligible E_{max} values another explanation is more plausible. An intravascular separation depending on different diffusibilities of the tracer molecules results in an initial lag of the more diffusible molecule (sodium) This convection-diffusion effect, described by Taylor (1953), also gives a late and apparent positive extraction and a typical U-shape of the E curve. In studies of the brain capillaries Yudilevich & De Rose (1971) using hemoglobin as reference tracer, found E_{max} for sodium between 0.25 and 0 68 while the E_{net}-values were around zero They considered the capillaries to be impermeable to sodium and concluded that because of Taylor diffusion the use of a protein as reference was not reliable. In the present study an U-shaped E_{net}-curve was found in the two experiments with high E_{max}, but also in most of the other expts. although less pronounced. Interlaminar diffusion may occur in the intravascular compartment and in the catheters used for injection and venous blood sampling. The effect is enhanced at low flow rates (Renkin 1959) We therefore used short catheters and considered this to be particularly important for the retinal vein catheter because of the low blood flow rate. In one experiment with a 5 cm long catheter which had a long gradually tapered tip we found an E_{max} of 0.80. An E_{net} of −0.12 again indicated Taylor diffusion, and stresses the importance of short catheters. The catheters used in expts. 77 188 and 77 234 did not differ in any respect from those used in the other expts

Our data thus support the hypothesis that there is a common barrier that excludes both albumin and sodium during a single passage through the retinal capillaries. This is in accordance with the interpretation of Yudilevich & De Rose (1971) in their studies on the permeability of the BBB. The results further support the suggestion that the blood drained by the venous plexus on the distal part of the optic nerve is mainly retinal blood. A significant contribution of choroidal blood should have been unmasked because of the high permeability of the choriocapillaris to sodium.

The BRB as well as the BBB can be disrupted by arterial infusions of hypertonic solutions (Rapoport 1976) In the experiment with hypertonic NaCl-solution we also found evidence for the sensitivity of the BRB to such treatment. An E_{max} of 0.39 was followed by an E_{net} of 0.30 indicating a true extravascular sodium loss

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Retinal and choroidal contribution to retinal metabolism in vivo A study in pigs

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The glucose metabolism of the retina was studied in vivo by determining glucose oxygen and lactate a-v differences for choroidal and retinal blood in pigs at different levels of intraocular pressure At normal intraocular pressure the choroidal a-v differences were 0.07 and 0.11 mmol/l for glucose and oxygen respectively The corresponding figures for the retina were 0.44 and 2.15 At increased levels of intraocular pressure the choroidal and retinal glucose and choroidal oxygen a-v differences increased, thus at least partly compensating for reduced blood flow A major part of the oxygen and glucose consumed by the retina was delivered by the choroid. The total amount of oxygen extracted from choroidal and retinal blood could only account for complete oxidation of 37% of the extracted glucose The results obtained in determinations of lactate a-v differences indicate that part of the remaining glucose is used for anaerobic glycolysis.

Key words Retinal metabolism glucose oxygen, lactate

The high glycolytic and respiratory rate of the isolated retina, and its remarkable ability to convert glucose into lactate even in the presence of oxygen was first demonstrated by Otto Warburg (1927) and has later been confirmed by several investigators (see Graymore 1970) Very little is known about retinal metabolism under in vivo conditions when influenced by factors such as blood flow, composition of the blood supplying the tissue and restrictions caused by barrier mechanisms between blood and tissue

Quantitative studies on the metabolism in vivo can be made if both the rate of blood flow and the a-v differences for metabolic substrates and metabolites are determined. The human retina depends on two vascular beds the retinal capillaries and the choriocapillaris. In pigs, with a similar vascular supply to the retina, it is possible to obtain venous blood from these two sources by cannulating a venous plexus around the optic nerve and one vortex vein (Törnquist, Alm & Bill 1979)

The purpose of the present study was to estimate aerobic and anaerobic glucose metabolism of the retina in vivo at normal and high IOP and the roles of the retinal and choroidal vessels by determining the a-v differences for oxygen glucose and lactate. The blood flow values needed in these calculations were obtained in a previous study (Törnquist Alm & Bill 1979)

METHODS

Two series of experiments were performed.

A The surgical procedure has been presented elsewhere (Törnquist, Alm & Bill 1979) and will only be summarized here.

14 pigs of both sexes weighing between 19.5 and 27.5 kg were anesthetized, tracheotomized and if needed artificially ventilated One femoral artery was cannulated for measurements of the MAP and one vein for infusions. The retinal venous plexus surrounding the optic nerve at its entrance to the globe was exposed and this venous plexus as well as one vortex vein, were cannulated In the same eye the anterior chamber was cannulated with two steel cannulas, one connected to a pressure transducer and the other to a reservoir containing mock aqueous

Abbreviations. BBB = blood-brain barrier BRB = blood-retinal barrier RPE = retinal pigment epithelium, IOP = intraocular pressure, MAP = mean arterial blood pressure and PP = perfusion pressure (PP = MAP − IOP).

Table 1 *The a v differences for glucose oxygen and lactate for retinal and choroidal blood at normal IOP*
Note that in calculations of the means expts. 138, 219 224 and 234 were calculated See text For lactate the present mean value was calculated for series B The values are given in mmol/l

	Glucose		Oxygen		Lactate	
Expt.	Choroid	Retina	Choroid	Retina	Choroid	Retina
Series A						
111	0 01	0 29	0 19	1 41		
117	0 09	0 58	0 00	0 96		
122		0 32				
131		0.53				
134		0 76		1 82		
138			0 16	3 92	−0.46	−0.46
161	0 08	0 65	0 13	2 41	0 06	0.06
181	−0 02	0.54	0.20	2.77	−0 06	0.02
188	0 04	0 04	0 05	01	0.04	−0.10
216	0 05	0.33	0 11	2 81	−0 17	−0.07
219	0.22	0 68	0.28	2.84	−0 75	−0 67
224	0 49	0 74	0.31	3.29	−0 39	−0.32
229	−0 03	0 39	0 06	3 01		
234	0 20	0 42	0 12	2.78	−0 62	0 49
Series B						
184	0 15					
221	0 14				−0.10	
223	0 19				−0.04	
306					−0.16	
307					0.05	
382					−0 10	
Mean	0 07	0 44	0 11	2 15	−0 07	
S E	0 02	0 07	0 03	0 76	0.04	
n	10	10	7	8	5	
P<	0 02	0 001	0 01	0 001	n s	

humor for artificial control of the IOP In order to assure that the blood sampled from the retinal venous plexus was mainly retinal and that the endothelial part of the BRB was intact the sodium extraction from the retinal vascular bed was determined with the single injection technique (Törnquist, Alm & Bill 1979)

Blood analyses

For oxygen saturation and glucose and lactate concentrations 5 samples were taken from arterial and from venous blood Only results where all 5 determinations were within 5% of the mean were accepted The mean values of the 5 determinations were used for calculating the a- differences

Hemoglobin concentration Two samples of arterial blood were analysed with a spectrophotometric cyanmethemoglobin method

P_{O_2} and pH One arterial sample was analysed with an automatic analyser (ABL 1 Radiometer Denmark)

Oxygen saturation was determined with an oximeter (Hellige microoximeter American Optical Company Buffalo N Y) The oxygen content in mmol/l was calculated according to the expression.

(Hb (g/l) × fractional oxygen saturation × 1.34)/22 41

In calculations of the a-v differences the amount of physically dissolved oxygen was neglected since such corrections using the hemoglobin-dissociation curve for pig blood published by Barteris & Harms (1959) never accounted for more than 3% of the a v difference calculated from data for oxyhemoglobin

Glucose concentration was determined with a conventional technique (Glox KABI Sweden)

Lactate concentration was fluorometrically determined with an enzymatic method (Lowry & Passonneau 1972 25 μl blood was sampled in glass capillaries and precipitated in 50 μl chilled perchloric acid The time between the start of sampling and the precipitation varied between 10 and 60 s and was the same for blood from all three sources Thus blood from the source with the lowest blood flow was precipitated immediately after sampling while blood from the other two sources was kept at room temperature until the same time had elapsed. The samples were stored at −70°C until analyzed

B In these expts we tried to further minimize the trauma to the eye by collecting only vortex blood. A-v differences for glucose and lactate were determined This was done in 6 pigs of similar weights and ages as in series A. The femoral arteries were cannulated one for blood sampling and the other for measurements of blood pres-

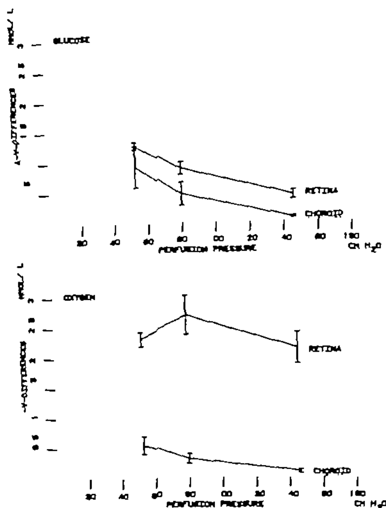


Fig. 1. Retinal and choroidal a-v differences (Mean±S.E.) at three different levels of PP 115–180, 70–90 and 40–65 cmH_2O respectively. Median values for PP are used. The group at PP 115–180 corresponds to the values presented in Table 1. (*a*) Glucose PP 70–90 and 40–65 n=5. (*b*) Oxygen. PP 70–90, n=5. PP 40–65 n=5 (retina), n=4 (choroid).

... One vortex vein was cannulated with standard surgical ... A small radial incision was made in the conjunctiva, and one extraocular muscle was divided longitudinally in order to expose the vein without cutting any vessels. Alternating samples were taken from arterial and venous blood.

Statistics

In the following mean and S.E. are given. Student's *t*-test was used to determine statistical significance. Significance levels indicate the probability that the true value is zero.

RESULTS

Arterial concentrations of glucose, oxygen and lactate were 3.69±0.26 (n=16), 6.16±0.25 (n=12) and 2.24±0.19 (n=13) mmol/l respectively. Table 1 shows the a-v differences obtained at normal IOP. The mean values are calculated for all animals except nos. 138, 219, 224 and 234 (see Discussion). Glucose and oxygen extractions were larger for retinal than for choroidal blood. Negative a-v differences for lactate were obtained for both retinal and choroidal blood in most experiments. In 8 animals where both retinal and choroidal lactate a-v differences were obtained, the a-v differences from both sources were of the same magnitude.

In one experiment of series B (no. 382), in which the blood flow through the vortex vein was de-

termined and 6 calculations were made of the a-v difference for lactate (each calculation was based on 5 determinations of arterial and choroidal lactate concentration) the flow was about 300 μl/min and the a v difference was -0.10 ± 0.01 mmol/l ($n=6$ $P<0.001$)

Fig 1 *a* and *b* show the values for glucose and oxygen obtained at different levels of IOP and the median value for PP at the time of blood sampling At reduced levels of PP the a-v differences for retinal and choroidal glucose and choroidal oxygen tended to increase In 5 expts where the a v differences were determined at normal and high IOP the increase in a v differences were 0.77 ± 0.16 and 0.91 ± 0.29 mmol/l for retinal and choroidal glucose respectively and 0.35 ± 0.10 mmol/l for choroidal oxygen The increments were significant at $P<0.05$

In a previous study (Törnquist Alm & Bill 1979) blood flow values for choroid and retina were obtained 1 732 and 64 mg/min respectively These values were used to transform the mean a-v differences presented in Table 1 to values for glucose and oxygen extractions in nmol/min The glucose extraction was 121 and 28 nmol/min from choroidal and retinal blood respectively and the corresponding figures for oxygen were 191 and 138 nmol/min Thus the choroid delivered about 80% of the glucose and 60% of the oxygen consumed by the retina.

DISCUSSION

A v differences for lactate

Table 1 shows that in 4 pigs of series A (expts 138 219 224 and 234) the production of lactate was considerably higher than in the remaining 4 In series B where surgery was minimized to that needed to cannulate a vortex vein all values were low Local hypoperfusion due to the more extensive surgery therefore seems to be a likely explanation for the high negative lactate a-v differences found in expts 138 219 224 and 234 Consequently these experiments were not included for calculations of "normal" a v differences on which the discussion of retinal metabolism *in vivo* is based These expts are of considerable interest however since they demonstrate that during marked lactate production in the retina high lactate concentrations were observed in both retinal and choroidal venous blood

Retinal a-v differences

Blood sampled from the retinal venous plexus fulfils the criteria for pure retinal blood (Törnquist Alm & Bill 1979) and the a-v differences can thus be assumed to be caused by exchange with retinal tissue In the present study highly significant a-v differences were found for oxygen and glucose. To our knowledge no previous study has been published where direct cannulation of a retinal vein has made it possible to determine oxygen and glucose contents of retinal venous blood The oxygen saturation of retinal venous blood in humans has been estimated from fundus photographs by a photometric method (Hickam & Frayser 1966) and the reported mean value 59% is similar to the observed mean value in the present study 56%

Previous studies have shown that the retinal capillaries like the capillaries in the brain (Yudilevich & De Rose 1971) restrict passive diffusion of low molecular weight water soluble substances (Törnquist Alm & Bill 1979) The rather marked a-v difference for glucose in the present study indicates that there is a carrier mechanism involved in glucose transport over the capillary membrane In the brain there is a stereo-specific glucose carrier system (Crone 1965) and this has also been suggested for the retinal capillaries (Dollery Henkind & Orme 1971)

Uveal a-v differences

The vortex veins may also carry some blood from the anterior uvea. Thus the measured a-v differences may differ somewhat from true choroidal values In cats where a better separation of choroidal and anterior uveal blood is possible the a-v difference for oxygen in the anterior uvea is about 1-4 times that of the choroid (Alm & Bill 1970) If the same is true in pigs where blood flow through the anterior uvea normally is about 20% of total uveal blood flow the observed oxygen extraction will overestimate the true choroidal value with 10% if all blood from the anterior uvea is drained by the vortex veins Törnquist (1979) found a choroidal a-v difference for glucose of 0 71 mmol/l in cats while no measurements were made for the anterior uvea

Effects of increased IOP on retinal and uveal a-v differences

The low choroidal a v difference for oxygen observed in the present study is similar to previously

Table 2. *Glucose and oxygen consumption in µmol/min per 100 g tissue*

The values calculated for the present study are based on wet weight for the retina of 135 mg. Values for the retina *in vitro* and the brain obtained by other investigators are included for comparison

	Present study	Rat retina in vitro (*a*)	Young men brain (*b*)	Cerebral cortex rat (*c*)
Glucose consumed	110	187	31	91
Oxygen consumed	224	226	156	491
Fraction of glucose not oxidized (*d*)	0.63	0.80	0.16	0.10

(*a*) Ames & Gurian (1963) [illegible] (1962).
(*b*) Sokoloff (1960).
(*c*) Norberg & Siesjö (1974).
(*d*) Calculated for oxygen and glucose consumption on the assumption that: (i) all oxygen is used for glucose oxidation, (ii) 6 moles of oxygen is used for oxidation of 1 mole of glucose.

[illegible] reported values for dogs (Cohan & Cohan 1963) and [illegible]s (Alm & Bill 1970). In the study on cats it was [illegible] that the reduction in choroidal blood flow [illegible] an increase in IOP was partly compensated for by an increased extraction of oxygen [illegible] volume blood, which resulted in an unchanged oxygen extraction per min at moderate IOP increments. In the present study a similar observation was made for glucose extraction from both choroidal and retinal blood, and for oxygen extraction from choroidal blood (Fig. 1*a* and *b*).

Choroidal and retinal contribution to retinal metabolism

Part of the oxygen and glucose extracted from the choroidal blood is consumed by the choroid. The metabolism of the choroid is less well known than that of the retina, but Elliot, Teller & Beigelman (1965) have shown that the uptake of labelled glucose per wet weight tissue in the dog choroid is about 15% of that in the retina. In pigs the wet weights of the retina and the choroid are about 135 and 35 mg respectively. Thus the choroid can be expected to utilize less than 4% of the glucose consumed by the choroid and the retina together.

The oxygen consumption of the dog choroid has been studied *in vitro* (O'Rourke & Benson 1970). The reported value 0.73 µl/mg dry weight per h, corresponds to 4 nmol/min consumed by pig choroid in the present study or about 1% of the total oxygen consumed. Therefore no corrections have been made for choroidal metabolism in the following calculations.

In a study on cats (Alm & Bill 1972) it was estimated that about 80% of the oxygen consumed by the retina was delivered by the choroid. Also in pigs the choroidal blood flow plays an important role in the nutrition of the retina. In the present study about 60% of the oxygen and 80% of the glucose consumed by the retina were delivered by the choroid. These calculations are based on the observation that choroidal blood flow is about 27 times that through the retina (Törnquist, Alm & Bill 1979). The observed choroidal a-v difference for glucose was small and with a considerable variation, but even if we assume that the true choroidal a-v difference for glucose is at the lower limit of the 95% confidence interval the calculated amount of glucose delivered by the choroid makes up 65% of the total retinal glucose consumption.

Retinal metabolism in vivo

The calculated total amount of glucose and oxygen delivered to the retina from the choroidal and retinal circulations are 149 and 329 nmol/min respectively at normal PP. These figures, transformed to consumption per min per 100 g tissue are presented in Table 2 together with values reported for the retina *in vitro* and the brain *in vivo*. Both oxygen and glucose consumption of the retina are larger than the values for mixed brain tissue. The values reported for the rat cortex show that there are marked differences within the brain although species variation may have contributed (Norberg & Siesjö 1974).

The calculated oxygen consumption in the present study is of the same order as that observed *in vitro* while the glucose consumption is lower. In

both studies however the amount of oxygen is insufficient for complete oxidation of the extracted glucose The fraction of glucose that cannot be completely oxidized is much larger than in the brain Part of the discrepancy between retinal glu cose and oxygen extractions may be explained by other uses for glucose such as glycogen and amino acid synthesis and the pentose phosphate pathway In a study *in vitro* Reading & Sorsby (1962) found no evidence for glycogen synthesis while 3–4% of the glucose was used for synthesis of amino acids About 15% of the glucose is used for the pentose phosphate pathway (Graymore 1969) It is also possible that part of the discrepancy between glucose and oxygen extractions is due to a considerable fraction of glucose being used only for anaerobic glycolysis *in vivo* as it is *in vitro* In the present experiments with a minimal surgical trauma lactate production varied considerably and was not statistically significant This might be due to methodological difficulties or a true variation be tween the animals To establish if a significant lactate production may occur at undisturbed circu lation we made repeated lactate determinations in one pig (exp 382) In this particular expt a highly significant negative a v difference of −0 10 mmol/l was obtained A small part of this difference may be due to lactate production in the lens (see Cotlier 1975) the cornea (see Graymore 1970) and the vessels of the choroid (see Hellstrand 1976) This figure −0 10 mmol/l corresponds to about 130 μmol/min per 100 g tissue a figure that would be obtained if about 60% of the extracted glucose was used for anaerobic glycolysis in the present study Although the figure only represents one pig, it suggests that the discrepancy observed in the whole material for glucose and oxygen extractions can partly be explained by lactate production *in vivo* In their study *in vitro* Reading & Sorsby (1962) found a lactate production that to a large extent explained the discrepancy between the glucose and oxygen consumption presented in Table 2.

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Effect of vagus nerve stimulation on the secretory-granule volume of the principal cells of the mouse gallbladder epithelium

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WAHLIN T., HULLIGER, M & AXELSSON H Effect of vagus nerve stimulation on the secretory-granule volume of the principal cells of the mouse gallbladder epithelium. Acta Physiol Scand 1979 106 359–363 Received 23 Jan 1979 ISSN 0001-6772. Departments of Pathology and Physiology University of Umeå, Sweden.

Experiments in mice were performed in order to investigate whether vagal activity could affect glycoprotein secretion from gallbladder principal cells. This cell type was studied with the electron microscope in control animals and after electric stimulation of the right or left nervus vagus. The volume density of glycoprotein containing granules was determined using morphometry It was found that stimulation of the left vagus nerve significantly reduced the relative cellular volume of secretory granules in the principal cells of the gallbladder Right vagus stimulation was accompanied by a weak but insignificant increase in secretory granule content. It is suggested that the left vagus nerve may exert a direct influence on glycoprotein secretion from gallbladder principal cells

Key words Mouse gallbladder principal cells, glycoprotein content, nervus vagus, electrical stimulation, morphometry

Previous studies of the mouse gallbladder mucosa have established that the principal cells of the gallbladder epithelium contain secretory glycoproteins (Wahlin et al. 1974). Increasing attention has been paid to the medical importance of these substances as they have been implicated in gallstone formation (Womack et al 1963 Bouchier et al 1965, Freston et al. 1969 Wahlin 1976). However the physiological role of these glycoproteins is still obscure, as are the factors governing the release of these proteins into the bile The gastrointestinal hormone cholecystokinin-pankreozymin (CCK PZ) induces an increased secretory activity both in vivo and in vitro, suggesting that this hormone may also control physiological secretion of gallbladder glycoproteins (Wahlin et al 1975 Wahlin et al. 1976). Furthermore stimulation in vivo and in vitro with the cholinergic agent pilocarpine has resulted in an increased secretion of glycoproteins from the mouse gallbladder principal cells (Wahlin et al. 1977 Axelsson et al. 1978, 1979).

These observations are in agreement with the histo- and cytochemical findings that cholinergic nerve fibres appear very close to the mouse gallbladder principal cells (Wahlin et al 1977) The parasympathetic innervation of the gallbladder is supplied by the vagus nerves (for ref ses e.g. Mester 1953 Belli et al. 1956, Burnett et al 1964 Wallraff 1969).

However the literature does not contain any reports on the effects of electrical stimulation of the vagus nerve on the release of glycoprotein granules from the gallbladder principal cells

In the present study ultrastructural changes in the mouse gallbladder principal cells were investigated following electrical stimulation of the right or left vagues nerve in the cervical region. To this end a quantitative electron microscopic investigation of the principal cells was carried out. In particular any change in the relative cellular volume of secretory granules was determined. It was found that electrical stimulation of the left but not the right vagus nerve evoked an appreciable (40 %) reduction of the granule content of the principal cells

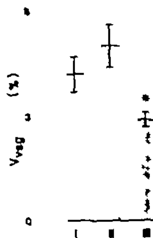


Fig 1 Volume density (V_{vsg} in percent) of secretory granules of mouse gallbladder principal cells in control animals (I) and after stimulation of the right (II) or *left* (III) vagus nerve Mean and S E for groups of 7 animals are indicated by the height of the columns and the vertical bars $p<0.05$



Fig 2 Electron micrograph of a mouse gallbladder principal cell from a control animal In the apical portion of the cell numerous secretory granules (*S*) are present Volume density of secretory granules (V_{vsg}) in this cell 4.5% Marker 1 μm

MATERIAL AND METHODS

Animals Black mice of both sexes (weighing 2.–.8 g) were used for the present expts The animals were 6–7 months old and taken from a locally bred colony (C57BL strain) Prior to the expts they were kept under normal laboratory conditions with free access to water and a standard pellet diet (Astra-Ewos Södertälje Sweden)

Electrical stimulation of vagus nerve 1 black mice from the same colony were used They were divided into 3 groups of equal size (group I controls group II right vagus stimulation group III left vagus stimulation). The animals were anesthetized by i p injection of pentobarbital sodium (4 mg/100 g b wt) Control animals were killed after anesthesia and the gallbladder was immediately dissected out and prepared for fixation For each of the remaining animals one of the vagal truncs was carefully dissected from the surrounding tissue at the cervical level The proximal end of the freed segment was crushed and tied off and the nerve was then mounted on silver wire electrodes surrounded by liquid paraffin Electrical stimulation at 50 Hz, with a pulse duration of 5 ms was carried out with the intensity of stimulation ranging between 1 and 10 V The threshold for the negative chronotropic action of vagus stimulation on the heart was determined routinely and the intensity of stimulation was always adjusted to twice threshold Electrical stimulation was performed over a period of 10 min which was divided into alternate intervals of excitation and rest each lasting 30 s

The cardiac action of vagus nerve stimulation was assessed by the ECG which was recorded with silver wire electrodes placed on the skin of the thorax The skin was routinely moistened with Ringer solution. The signals were amplified with a standard differential amplifier and continuously displayed on a monitor screen Following the period of vagus nerve stimulation the animals of groups II and III were immediately sacrificed and their gallbladder was removed

Tissue preparation Gallbladders from controls and vagus-stimulated animals were rapidly transferred to 1% osmiumtetroxide in 0.1 M phosphate buffer (pH 7.4) for fixation and kept in this solution for two hours at 4°C Subsequently they were rinsed for two hours in 0.9% NaCl The specimens were then dehydrated in graded ethanol solutions and propylene oxide and subsequently embedded in Epon 812 Sections were cut at a feeding rate of 70 nm on an LKB Ultrotome using glass knives and collected on naked copper grids. After contrasting with uranyl acetate and lead citrate the sections were examined in a Siemens Elmiscope I A electron microscope

Stereological and statistical methods For the volumetric analysis of the secretory granules (V_{vsg}) a point counting method was used (Weibel & Elias 1967 Weibel et al 1969). For the estimation of the volume density of secretory granules the values of granule volume (V_{vsg}) expressed in relation to the volume of the cytoplasm of the principal cells The planar area of the whole cells was also estimated About 15 principal cells per mouse were photographed from coded grids and quantitative electron microscopic measurement were then taken from 10 randomly selected cells the only prerequisite being that the cells displayed a nucleus and an apical as well as a basal surface A multipurpose test screen with 1 cm lines was randomly placed over each micrograph and the number of end points lying over the secretory granules and the whole cells were counted V_{vsg} was then estimated by di[illegible]

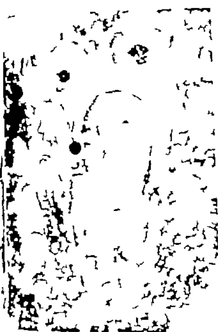


Fig. 3. Principal cell after electrical stimulation of left vagus nerve. Only a few secretory granules (S) are seen in the apical portion. V_{vsg} in depicted cell 2.5%. Electron micrograph. Marker 1 μm.



Fig. 4. Mouse gallbladder principal cell after electrical stimulation of right vagus nerve. Increased amounts of secretory granules (S) in the apical and subapical portions of the cell. V_{vsg} in this cell 7.5%. Electron micrograph. Marker 1 μm.

number of points falling over the glycoprotein granules to the total number of points falling over the cytoplasm. The planar area was also calculated by point-counting. Unpaired Student's *t*-test was used in order to assess whether electrical stimulation of the right respectively left vagus nerve was accompanied by significant changes in the morphometric parameter. The stereological and statistical methods and their possible errors have been discussed in detail in an earlier paper (Wahlin et al. 1976).

RESULTS

Electrical stimulation of either branch of the vagus nerve reduced the heart rate very drastically (by about 80%). During right vagus stimulation the mean and standard deviation of the animals' averaged heart rate were 47/s±23. During the rest periods the values were 214/s±38. During left vagus stimulation the mean heart rate was 43/s±8 (S.D.), as compared with 230/s±39 during the periods of rest. Thus the electrical stimulation was equally effective in the two test groups.

In contrast, the effects of vagus stimulation on the secretory-granule *volume* of the gallbladder principal cells differed markedly for the two sides. The results of the quantitative electron microscopic analysis of the secretory-granule volume are summarized in Fig. 1. After 10 periods of 30 s stimulation of the left vagus the volume density of the secretory granules was significantly diminished from 4.2%±0.5% (S.E.) to 2.9%±0.2 (S.E.) ($P<0.05$, $n=7$). In contrast, after the same stimulation of the right nerve the volume density of secretory granules was slightly increased to 5.0%±0.6 (S.E.), but the difference to the control values (4.2%±0.5) was not significant ($p>0.05$). The direct comparison of the values of granule density following right and left vagus stimulation also exhibited a significant difference ($p<0.01$). There was no significant change in the planar area of the stimulated cells (78.6 μm^2±4.5 (S.E.), left vagus; 90.8 μm^2±5.9, right vagus) as compared with the control cells (93.0 μm^2±5.6; $p>0.05$ in either case).

In Figs. 2 to 4 examples of the effects of vagus stimulation on the ultrastructural *morphology* of the principal cells are illustrated. Cells from control animals are illustrated in Fig. 2, showing the normal

frequency of secretory granules (cf Wahlin et al 1976*a* Axelsson et al 1979) Stimulation of the left vagus resulted in a clearly decreased density of secretory granules in most cells (Fig 3) Following electrical stimulation of the right vagus many cells showed some increase in the number of secretory granules (Fig 4) The Golgi area as well as the endoplasmic reticulum seemed to be unaltered after stimulation of either branch of the vagus nerve (Figs 3 and 4) as suggested by the comparison with the illustrated control cells (Fig ?)

DISCUSSION

One of the principal functions of the gallbladder epithelium is the concentration of bile by selective absorption of water inorganic ions and small amounts of bile salts These processes are well known and they have been extensively studied On the other hand little attention has been paid to the secretory ability of these cells From previous studies it is evident that the mouse gallbladder principal cells store glycoproteins in cytoplasmic granules which are discharged into the bile by ex ocytosis (Wahlin et al 1974 Wahlin et al 1975) A basal secretion of these proteins takes place ir respective of the animal s nutritional state (Wahlin et al 1976*a*) In recent studies factors which might regulate the glycoprotein secretion from the princi pal cells were investigated It was found that gastric deposition of olive oil and in vivo and in vitro stimulation with the gastrointestinal hormone cholecystokinin pancreozymin (CCK PZ) induce an increased secretory activity However apart from humoral factors the glycoprotein secretion seems also to be subjected to neural influences This was suggested by the finding that the cholinergic drug pilocarpine caused an increased release of glycoprotein containing granules from the principal cells both in vivo and in vitro (Wahlin et al 1977 Axelsson et al 1978 1979)

The present study shows that direct electrical stimulation of the left vagus nerve causes a release of secretory granules from the mouse gallbladder principal cells The present data are in agreement with previous findings that parasympathetic nerve fibres appear immediately adjacent to the mouse gallbladder principal cells and that cholinergic substances induce an increased secretion of glycoproteins from these cells (Wahlin et al 1977 Axelsson et al 1979) Thus it seems that the simplest explana tion of the present findings is that the left vag nerve has a direct secretory effect on the princi cells These cells are probably supplied w acetylcholine receptors (Axelsson et al. 1979). contrast stimulation of the right vagus nerve not cause a significant change in the size of t secretory granule population of the principal cel In fact a slight increase in the size of the populati was found This might be attributable to a reducti of the basal secretion due to a decrease of the bl supply to the gallbladder This seems quite pr able since the cardiac action of right vagal stim tion was very pronounced and in fact of the sa size as with left vagal stimulation However detailed mechanisms of right vagal action remai matter of speculation On the other hand as rep the effects of left vagal stimulation it seems v unlikely that the increase of glycoprotein secre was simply due to a systemic effect on the bl supply since this should then have been companied by reduced secretion

The present data suggest that only fibres from left vagus nerve supply the gallbladder mucos the mouse Direct morphological confirmatio this interpretation is not available since the li ture seems not to contain any reports concern the details of the parasympathetic innervation of mouse gallbladder Yet the present suggestio supported by anatomical studies in other m malian species including man These showed the parasympathetic nerve supply of the gallbla is mainly derived from the left vagus trunk (cf for man McCrea 1924 Mitchell 1940 Mester Belli et al 1956 Siegfried 1960 dog McCrea Jemerin & Hollander 1938 Stavney et al 1963, McCrea 1924)

Gallbladder glycoproteins have been assoc with gallstone formation and it has been sugg that these substances may play an important ro the initiation of stone formation (Womack e 1963 Bouchier et al 1965 Freston et al Hultén 1970) Dietary induction of gallstone mice is preceded by an increased synthesis secretion of glycoproteins from the gallbla principal cells (Wahlin 1976*b* 1977) The kn edge of the factors regulating glycoprotein thesis and secretion under normal and patholo conditions is still limited Yet the present find suggest that parasympathetic activity mediate the left vagus nerve may directly control normal and pathological secretion of glycoprot

[illegible] directed neural action on the gallbladder [illegible] might be involved in the processes leading [illegible] the formation of gall stones

[illegible] work was supported by the Swedish Medical Re[illegible] Council, grant No 78-12X-4758-03 and by the Swiss [illegible] Foundation, M. H grant No 831 445 76. W [illegible] to thank Dr B Appelberg (Department of Physiolo[illegible], University of Umeå, Sweden) for providing laboratory [illegible] The skilled technical assistance of Mrs Elisabeth [illegible] and Mrs Gertrud Nilsson is gratefully ac[illegible]

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A study on the role of endogenous prostaglandins in the development of exercise-induced and post occlusive hyperemia in human limbs

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NOWAK, J. & WENNMALM, Å.: A study on the role of endogenous prostaglandins in the development of exercise-induced and post-occlusive hyperemia in human limbs. Acta Physiol Scand 1979, 106: 365–369. Received 30 Jan. 1979. ISSN 0001-6772. Department of Clinical Physiology, Karolinska Institute at Huddinge Hospital, Huddinge, Sweden.

The contribution of endogenous PGs to the development of functional (exercise-induced) and reactive (post-occlusive) hyperemia was investigated in healthy volunteers. Leg blood flow during dynamic leg exercise was estimated by an indicator dilution technique. Forearm blood flow during supine leg exercise and forearm and calf blood flow following 5 min of arterial occlusion were measured plethysmographically. All subjects were examined before and after pretreatment with indomethacin, a PG synthesis inhibitor. During leg exercise, and in the absence of indomethacin, a 10-fold rise in leg blood flow was observed. Forearm blood flow increased moderately. Both these blood flow effects of exercise were unaffected by indomethacin. Following arterial occlusion, marked hyperemia developed in the forearm and the calf. Indomethacin significantly reduced the magnitude of the reactive hyperemia both in the forearm and in the calf, decreasing both the peak value and the duration of the vasodilation. These data reveal differences between the mechanisms behind functional and reactive hyperemia in man, suggesting an appreciable contribution of endogenous PGs to post-occlusive vasodilation only.

Key words: Exercise, functional hyperemia, human limbs, indomethacin, prostaglandins, reactive hyperemia.

A number of endogenous vasoactive substances, circulating or local have been suggested as regulators of peripheral vascular resistance in various species (Haddy & Scott 1968, Altura 1971, Lewis 1971). In recent years endogenously synthetized prostaglandins (PGs) have been pointed out as possible mediators of peripheral blood flow regulation (Bergström 1972, Staszewska-Barczak & Vane 1975, Messina 1975, Nowak & Wennmalm 1978). In animal experiments, local PG-release has been shown to contribute both to functional and to reactive hyperemia (Herbaczynska-Cedro et al. 1974, Herbaczynska-Cedro & Vane 1974, Messina et al. 1977, Wenner et al. 1977). Similar results have been obtained in experiments with post-occlusive and exercise-induced hyperemia in the human forearm (Kilbom & Wennmalm 1976), suggesting that PG-dependent vasoregulatory mechanisms also occur in man. In the present study we attempted to further evaluate the role of endogenous PGs in vascular responses to exercise and ischemia in man by studying, in particular, vasodilation induced by exercise in active and inactive tissues, as well as post-occlusive hyperemia in forearm and calf.

MATERIAL AND METHODS

The study was performed with the permission of the Ethical Committee at the Karolinska Institute.

16 healthy volunteers, 5 women and 11 men, aged 20–40 years were studied. They were all informed about the aim and experimental procedure of the study before giving their voluntary consent to participate. All subjects were investigated twice, before and after pretreatment with indomethacin (Indomée, 1.25 mg/kg b.wt. rectally). 3 series of expts were performed. Some of the subjects participated in all three.

1. *Effect of indomethacin on leg blood flow during exercise*

In 6 subjects, all men, aged 24–32 years, a teflon catheter (outer diameter 1.2 mm) was inserted percutaneously under local anaesthesia into each femoral artery. A similar

Table 1. Total reactive hyperemia expressed in ml/100 ml tissue in forearm and calf measured in 10 subjects during the 180 s recovery period following 5 min arterial occlusion before and after administration of indomethacin

	PG synthesis intact	PG synthesis inhibited	% remaining after indomethacin
Forearm	10.1±1.5	4.3±1.2 ($P<0.01$)	42
Calf	13.0±1.5	8.2±1.4 ($P<0.01$)	63

test for paired observations was used for statistical analysis. All values are given as the mean±S.E. of the mean.

RESULTS

1. *Leg blood flow during exercise*

The resting leg blood flow was 0.4±0.03 l/min. Exercise elicited a successive marked increase in this flow (Fig. 1). Increasing work load was paralleled by an augmented flow. The maximal figure for blood flow (4.3±0.2 l/min) was reached at work load of 150 W implying more than a 10-fold rise above the resting level. Indomethacin did not affect resting leg blood flow neither did it change the flow-enhancing effect of dynamic work. The leg blood flow following administration of indomethacin almost exactly paralleled in the flow figures obtained before indomethacin (Fig. 1)

Forearm blood flow during leg exercise

After the onset of leg exercise the blood flow in the forearm increased continuously from the basal level (3.3±0.3 to a maximum of 5.4±0.7 ml 100 ml tissue min (Fig. 2) This increase corresponded to 64 % and was statistically significant ($p<0.01$) Indomethacin displayed no effect on the forearm blood flow during leg exercise.

3. *Post-occlusive hyperemia*

Five min of arterial occlusions was found to induce a prominent post-occlusive increase in flow both in the forearm and in the calf The post-occlusive period of hyperemia was brief within 3 min the flow rates had generally returned to the resting values both in the forearm and in the calf

In the forearm, the mean basal blood flow was 3.1±0.4 ml/100 ml tissue/min After the end of the 5 min occlusion a peak flow of 17.8± 2.3 ml 100 ml tissue min was recorded The mean flow recovery time was 165 s (Fig. 3) Indomethacin did not significantly affect the basal blood flow but markedly decreased the magnitude of the reactive hyperemia ($P<0.01$) The peak post-occlusive flow after indomethacin was reduced to 13.9±_.0 ml 100 ml tissue min and the flow recovery time to 90 s. The total forearm hyperemia following administration of indomethacin was 42% of the control value (Table 1)

In the calf the mean basal blood flow was 3.7±0.5 ml 100 ml tissue min After release of the occlusion, a peak value of 23.9±1.8 ml 100 ml tissue min was recorded. The duration of the post-occlusive calf hyperemia was 135 s (Fig. 4)

In contrast to its effect in the forearm, indomethacin reduced the resting calf blood flow ($P<0.05$) A significant ($P<0.01$) decrease in peak post-occlusive hyperemia, to 20.6±3.0 ml 100 ml tissue min was also obtained, as well as a

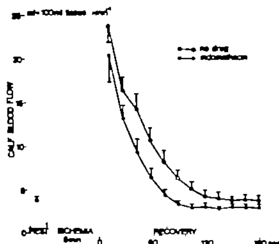


Fig. 4 Calf blood flow at rest and during the recovery period after 5 min arterial occlusion. Mean values and S.E. of the mean for 10 subjects before and after indomethacin.

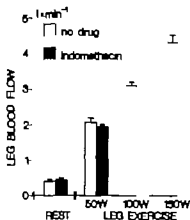


Fig 1 Leg blood flow at rest and during dynamic work Mean values and S E. of the mean for 6 subjects before and after indomethacin.

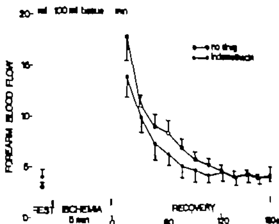


Fig 3 Forearm blood flow at rest and during the reco ery period after 5 min arterial occlusion. Mean values a S E. of the mean for 10 subjects before and after I domethacin

teflon catheter was inserted into the femoral vein of the leg to be investigated All catheters were directed proximally with their tips positioned at the level of the inguinal ligament Leg blood flow was estimated using the constant rate infusion technique (Jorfeldt & Wahren 1971) A solution of indocyanine green dye (Cardio-Green HW & D) was infused intraarterially into the investigated leg under simultaneous sampling of arterial and femoral venous blood Plasma dye concentration was determined spectrophotometrically After repeated blood flow measurements at supine rest, exercise in upright body position was performed using an electrically braked bicycle ergometer (Siemens-Elema Sweden) The subjects per formed a 6 min work at each of the loads 50 100 and 150 W Leg blood flow was measured 4 times at each work load The procedure was repeated 1 week later after 2 h pretreatment with indomethacin.

2 Effect of indomethacin on forearm blood flow during leg exercise

In 10 subjects 5 women and 5 men aged 20–40 years, forearm blood flow was measured at supine rest and during supine leg exercise by means of venous occlusi plethysmography The changes in forearm volu during proximal venous occlusion were measured wi an air filled thin-walled rubber plethysmograph c placed around the thickest part of the forearm (Dohn 19 Graf & Westersten 1959) The venous occlusion pressu was 60 mmHg and the pressure in the plethysmograp cuff about 3 mmHg. The pressure changes in th plethysmograph cuff induced by venous occlusion we measured with a pressure transducer (EMT 31 Siemens Elema, Sweden) and recorded by a Mingograf 3 (Siemens-Elema, Sweden) During the measurements c forearm blood flow arterial inflow to the ipsilateral ha was occluded with a sphygmomanometer cuff Calibratio was undertaken by inflating the plethysmograph cuff wi a known amount of air (? ml) Initially the measurement of basal flow were performed 4 or 5 times Subsequently leg work was performed for 10 min using an electrically braked bicycle ergometer as above The work load was 7 W for women and 100 W for men During the exercise forearm blood flow was measured every 2 min In domethacin was then given and the entire procedure wa repeated 2 h later

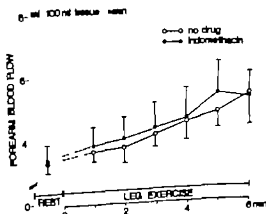


Fig 2 Forearm blood flow at rest and during dynamic leg exercise Mean values and S E of the mean for 10 subjects before and after indomethacin

3 Effect of indomethacin on post-occlusive hyperemia

Using the same groups of subjects and the same techni ques as above recordings of forearm and calf blood flow were performed at supine rest. After 4–5 measurements i basal state arterial occlusion was applied for 5 min on the upper arm or proximally on the thigh Postocclusion blood flow was measured every 15th s for 3 min after the end of the ischemic period Indomethacin was then given and the procedure was repeated 2 h later

4 Calculations

The total reactive hyperemia was calculated in each individual as the area between the basal blood flow level and the blood flow curve during recovery after arterial occlusion. The figures are expressed as ml/100 ml tissue St

development of reactive hyperemia. Ischemia has been shown to be a powerful stimulator of PG-synthesis and release (McGiff et al. 1970, Kent et al 1973, Herbaczynska-Cedro et al. 1974) and the inhibition of PG-synthesis has been demonstrated to significantly decrease reactive hyperemia in human forearm (Kilbom & Wennmalm 1976). The data presently display good agreement with these earlier observations and show in addition that a similar mechanism, although less pronounced quantitatively, operates in the calf. The results suggest that enhanced formation of endogenous PGs may be a common vasodilating mechanism in the development of reactive hyperemia in the human cardiovascular system.

In conclusion, the results presented here indicate that increased formation of PGs in the human vascular system is of minor importance for the development of exercise-induced vasodilation in active and inactive tissues. In contrast, the reactive hyperemia in skeletal muscle vasculature is mediated to a considerable extent by locally formed PGs.

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shortening of the hyperemia time to 105 s The total reactive hyperemia in the calf following indomethacin was 63 % of the control value (Table 1)

DISCUSSION

In the present study exercise induced hyperemia in working leg and resting forearm as well as post-occlusive hyperemia in the human forearm and calf were investigated before and after administration of indomethacin an inhibitor of PG biosynthesis (Vane 1971) The aim of these expts was to elucidate the role of endogenously formed PG in the development of different types of peripheral vasodilation

The dose of indomethacin used in the present study to inhibit the PG synthesis is sufficient to reduce the urinary excretion of the main PGE metabolite in man by 77–98 % (Hamberg 1972) which indicates that the endogenous PG-formation is inhibited to the corresponding extent

In the current expts dynamic work elicited a marked increase in leg blood flow which was unaffected by indomethacin This indicates that during leg exercise PG synthesis in the working muscle vasculature is not stimulated sufficiently to give rise to local concentrations of PG that may contribute significantly to the resulting vasodilation However evidence implicating exercise induced stimulation and release of PG-like substances into the venous effluent from working skeletal muscle has been demonstrated during as well as after exercise in dog hind limb (Herbaczynska-Cedro et al 1974) Furthermore in the human forearm indomethacin was found to diminish the hyperemia following dynamic work as well as the hyperemia developing in response to sustained isometric exercise (Kilbom & Wennmalm 1976) Finally in a recent study performed in our laboratory increased concentrations of radioimmuno-PGE material were demonstrated in the femoral venous blood during leg exercise in man (Nowak & Wennmalm 1978) All these data indicate that PG biosynthesis is in fact stimulated during muscle work The present finding that indomethacin did not affect leg blood flow during exercise may thus seem to conflict with some earlier reports

Although no obvious explanation for these contradictory results is available at present some alternatives should be considered Firstly most studies on the participation of endogenous PGs in the development of hyperemia have been p formed in animals (Herbaczynska-Cedro et al 19 Herbaczynska-Cedro & Vane 1974 Messina et 1977 Weiner et al 1977) in which the anesthesia intact animals) or perfusion condition (in perfu organs) may have depressed the normal v oregulatory mechanisms and thereby provided cess to others normally not operating. Secondly the human study referred to above (Kilbom Wennmalm 1976) the functional hyperemia duced by indomethacin was localized to forearm Consequently the results from that st do not conflict directly with those obtained h that tissue to-tissue differences do in fact occu also seen in the present expts where indometh reduced reactive hyperemia in the forearm to 4 but in the calf to only 63 % of the control value

In the present study an increase in forearm bl flow was observed after the onset of leg exerc Similar results with a transient increase in fore blood flow and a decrease in forearm vascula sistance during exercise of lower extremities l been reported earlier (Bevegård & Shepherd 19 Recently an increased level of plasma radi muno-PGE material was detected in the ve plasma sampled from the resting forearm during exercise (Nowak & Wennmalm 1978) Since PGs are potent vasodilators it is conceivable such vasodilation in the resting forearm was d enhanced vascular formation of PGs. However domethacin did not affect the increase of for blood flow in the present expts indicating other mechanisms are responsible for the vaso tion observed here In the present study leg cise was performed in supine body position elevated legs This body position and activi known to cause a shift of blood to the intratho venous bed resulting in stimulation of recepto the low pressure area of this vascular region (die et al 1957) This may serve as one o mechanisms behind the observed increas forearm blood flow The release of PGs observ this situation (cf above) is apparently of no oregulatory significance

Reactive hyperemia in the forearm and cal duced by 5 min of arterial occlusion contr sharply to the other two types of vasodil studied Thus indomethacin significantly redu both the peak and the duration of the post-occlusiv blood flow in both the forearm and the calf The data are consistent with a causal role for PG in th

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Substance P Release on trigeminal nerve stimulation effects in the eye

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ectrical stimulation of sensory nerves rather sur risingly causes release of mediators in the peripheral nerve endings in addition to that in central ones. In the periphery the release tends to cause signs of inflammation. One agent suspected of being released under these conditions and possibly involved in the related axon reflex mechanism is substance P (SP) (Lembeck 1953) an undecapeptide known to be present in a considerable proportion of the sensory neurons (Hokfelt et al. 1975) This hypothesis is supported by the observation that i.a. injection of SP causes responses similar to those of nerve stimulation (Lembeck Gamse & Juan 1977) as well as by the observation that stimulation of the mandibular nerve temporarily enhances the release of substance P—like immunoreactivity (SPLI) from the superfused tooth pulp in cats (Olgart et al. 1977). In the rabbit eye electrical or mechanical stimulation of the sensory nerves causes miosis in addition to signs of inflammation (Perkins 1957 Stjernschantz, Geijer & Bill 1979) Prostaglandins involved in some types of *ocular irritation e.g.* paracentesis seem to play no significant role in the response to nerve stimulation (Eakins 1977) The present paper provides evidence that SPLI is released into the aqueous humor of the eye when the trigeminal nerve is stimulated, that the release is not due to leakage from the blood vessels and that intracameral injection of SP causes miosis protein leakage from the blood vessels and a breakdown of the blood-aqueous barrier

METHODS

Experiments were performed in albino rabbits anesthetized with pentobarbital sodium. In all experiments one eye was subjected to stimulation whereas the contralateral eye served as control

In electrical stimulation, bipolar electrode was inserted under visual control into the exposed trigeminal nerve distal to the Gasserian ganglion After 4–6 min of electrical stimulation at 15 Hz, 10 V and 5 ms impulse duration the animal was killed by an i. Injection of saturated KCl. Immediately after death the aqueous humor of both eyes was quantitatively collected using plastic syringes.

Mechanical stimulation of the trigeminal nerve was performed at the same site as the electrical by rubbing or scratching the nerve for 1–2 min with pair of forceps. At 4–5 min later the animal was killed and the aqueous humor collected as described above In all stimulation expts. tubocurarine was used to produce muscular blockade A 20 µl sample was taken of each collected aqueous humor for protein analysis. The rest, 150–200 µl, was heated for 3–5 min at 100°C and then stored at −20°C until analysed. Aprotinine (Trasylol®) 500 KI E/ml was added to each plasma sample. After dilution 1 4 with saline plasma samples were heated and stored in the same way as aqueous humor samples. All plastic tubes except those for albumin analysis were pretreated with 0.5% albumin solution to prevent adsorption of SP to the plastic material. Radioimmunoassay was used to estimate the concentration of SPLI in plasma and aqueous humor (Nilsson et al. 1975) Immunoelectrophoresis (Laurell 1966) was applied to determine albumin concentration.

In experiments where the effect of intracameral injection of synthetic bovine SP (Peninsula laboratories, Palo Alto, California USA) was studied a branched needle was inserted into the anterior chamber of each eye. Bilateral injections of 10 µl of saline, on one side containing 0 1 or 1.0 µg of SP were carried out with precision syringes through one needle branch whereas the intraocular pressure was continuously registered with an electromanometer through the other Following the injection of a volume the corresponding amount was withdrawn after few seconds. 25 min after the intracameral injections 1.5 ml of a 2.0% solution of Evans blue was given i. After another 5 min the animal was killed by the i. administration of pentobarbital. Both eyes were transilluminated using fibre optics and a rough estimate was made of the state of the ciliary processes with regard to the amount of Evans blue. The aqueous humor was then collected from both eyes and analysed for protein content using the Amidoschwartz method (Munk Plum, Henriksen & Petersen 1954). In-

induced miosis within 1 2 min. 1 μg caused a pressure rise in all eyes, starting 10–15 min after the injection and peaking around 25 min The mean increase, without indomethacin, was 23.2 cmH_2O range 4–42 (n=5) With indomethacin it was 11.2 cmH_2O, range 6–20 (n=5). With 0.1 μg SP the corresponding figures were 17.5 cmH_2O range 3.5–30 and 9 and 1.5 cmH_2O range 0–4 (n=5) respectively The eye pressure before the injection of SP was [illegible]±3.7 cmH_2O (n=20) In all control eyes the eye pressure remained constant or decreased slightly Examination of the eyes after death showed marked leakage of Evans blue into the stroma of the ciliary processes in all SP treated eyes indicating protein leakage. A few ciliary processes with leakage were observed in several of the control eyes with a marked leakage only in two. Fig. 2 shows that there was considerable protein flux into the aqueous humor in all eyes receiving 1 μg SP The leakage normally produced with 0.1 μg SP appeared to be reduced by indomethacin

The fact that indomethacin did not prevent the effects of 1 μg SP indicates that they were not due to local irritation of the structures around the anterior chamber with release of prostaglandins (Eakins 1977). There can thus be little doubt that SP was the agent causing the contraction of the sphincter muscle of the iris and the increased capillary permeability It seems likely that the breakdown of the blood aqueous barrier constituted by the non-pigmented cells of the ciliary epithelium may have been secondary to vascular damage (Stjernschantz, Geijer & Bill 1979) Some involvement of the prostaglandins in this breakdown is suggested by the observation that indomethacin tended to prevent protein leakage from the ciliary processes and the rise in eye pressure at low concentrations of SP The contralateral response with respect to capillary permeability that was observed in some expts might be secondary to stimulation of the sensory nerves by SP and a consensual effect, or might be due to the trauma involved in the cannulation of the eye.

The results are in accordance with the hypothesis that one agent causing irritation in the eye when the trigeminal nerve is stimulated may be SP and suggest that the rabbit eye may be useful in further studies on the mechanisms involved in SP release The retina is known to contain considerable amounts of SP (Dunér Euler and Pernow 1954) The distribution within the anterior uvea is not known Previous (Olgart et al 1977) as well as the present results make it reasonable to assume that the neurons releasing SPLI on antidromic stimulation use SP as a transmitter substance.

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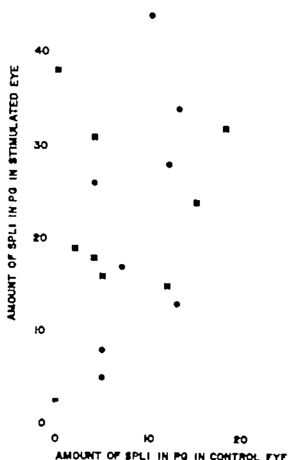


Fig 1 Effects of intracranial stimulation of the trigeminal nerve on the amount of substance P-like immunoreactivity (SPLI) in the aqueous humor ● 4–6 min electrical stimulation ■ 1–2 min mechanical stimulation

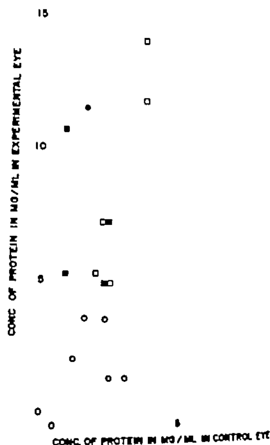


Fig 2 Concentrations of protein in the aqueous humor min after unilateral intracameral injection of substance with or without indomethacin given intravenously ● 0 µg SP no indomethacin ○ 0.1 µg SP indomethacin mg/kg b w ■ 1 µg SP no indomethacin □, 1 µg S indomethacin 20 mg/kg b w

domethacin 20 mg/kg b w was used in some expts to prevent formation of prostaglandins in the eye It was injected i.v 15–30 min before the injection of SP

RESULTS AND DISCUSSION

Electrical and mechanical stimulation of the trigeminal nerve Both types of stimulation caused ipsilateral miosis and usually a 5–6 mmHg drop in blood pressure from a resting level of about 75 mmHg Fig. 1 shows that in most expts the amount of SPLI in the aqueous humor of the stimulated eye was much higher than that in the control eye The mean difference (± S E.) in SPLI content between the two eyes was 13 1±3 6 pg ($n=10$ $P<0\ 01$) with electrical stimulation and 16 6±3 9 pg ($n=$ $P<0\ 005$) with mechanical stimulation The concentration of SPLI in the plasma was 66±7 pg/ml This value is higher than that in the control eyes which was about 40 pg/ml but lower than that in the stimulated eyes which was about 120 pg/ml. It i clear then that most of the SPLI appearing in th aqueous humor was not derived from the plasma a result of increased permeability of the blood aqueous barrier Occasional high SPLI values in th control eyes may have been due to consensual ef fects of the stimulation (Stjernschantz, Geijer & Bi 1979) The albumin in the aqueous humor wa measured on both sides to determine if in fac there was any appreciable breakdown of the barri during the short period of stimulation used Th concentrations were 2 9±0 6 mg/ml ($n=8$) in th electrically stimulated eyes and 1 7±0.5 mg/ml i the controls With mechanical stimulation the co responding figures were 2 1±0 4 and 2 0±0 6 mg/m ($n=7$) The mean plasma concentration of albumi in all experiments was 44±3 mg/ml

Effects of intracameral injection of SP In al expts injections of SP into the anterior chambe

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Delayed hypoperfusion in the cerebral cortex of the rat in the recovery period following severe hypoglycemia

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In the course of a study of mechanisms contributing to cell damage in the brain during hypoglycemia we have measured cortical metabolites in animals in which the hypoglycemia was sufficiently severe to cause cessation of spontaneous EEG activity as well as in animals in which recovery was induced by glucose administration (Agardh et al 1978). After 30 min of isoelectric EEG glucose injection was followed by a slow but relatively extensive recovery of cerebral metabolic state. However since there was a sustained increase in tissue lactate concentration and in the lactate/pyruvate ratio and incomplete recovery of the size of the adenine nucleotide pool, we tentatively concluded that some cell damage had been incurred.

Available evidence indicates that cerebral circulation (CBF) is upheld even in severe hypoglycemia (Kety et al. 1948, Della Porta et al. 1964, Norberg & Siesjö 1976). Furthermore since cellular redox systems seem to become oxidized (Lewis et al 1974) there are reasons to believe that cerebral oxygen supply is upheld and that cellular hypoxia should not contribute to the cell damage after hypoglycemia. However we have *now observed that* restoration of glucose supply is followed by a progressive decrease in local CBF. The present manuscript represents a preliminary account of these findings.

Male Wistar rats were starved for 24 h before being injected with insulin (Novo Actrapid®) in a dose of 40 IU kg⁻¹ i.p. They were then anesthetized with 2–3% halothane, tracheotomized, immobilized with tubocurarine chloride (1.5 mg kg⁻¹ i.v.) and connected to a respirator delivering nitrous oxide-oxygen (3:1). Femoral arterial catheters were inserted for blood pressure recording and blood sampling, and a femoral vein was cannulated for infusions. Goldplated screws were inserted in the skull bone for frontoparietal recording of EEG. Arterial P_{CO_2} was adjusted to 35–40 mmHg and body temperature was maintained close to 37°C.

Local cerebral blood flow (l-CBF) was measured with the autoradiographic ¹⁴C-iodoantipyrine technique of Sakurada et al. (1978) as described in detail in a previous communication (Abdul-Rahman et al. 1979). Measurements were performed in animals in which the spontaneous EEG activity had ceased for 30 min, as well as in those that had been allowed recovery periods of either 5, 30 or 90 min. Recovery was induced by i.v. injection of 0.5 ml of a 50% (w/v) glucose solution in saline followed by i.v. infusion (1 ml/h).

At the time of the l-CBF measurement, all hypoglycemic and posthypoglycemic animals had mean arterial blood pressures exceeding 120 mmHg, arterial P_{O_2} values higher than 85 mmHg, and arterial P_{CO_2} values in the range 22–35 mmHg (hypoglycemic animals) or 31–46 mmHg (post-hypoglycemic animals). Fig. 1 shows the l-CBF values in frontal, parietal and auditory cortex. At the end of the 30 min period of ceased EEG activity all three cortical regions showed marked hyperemia. Following glucose administration local flow rates progressively fell and after 90 min l-CBF in frontal and parietal cortex was reduced to 30–40% of control. In the auditory cortex CBF fell even further, the *values being close to 20% of control. Comparable* reductions were observed in other structures, e.g. in the hippocampus (not shown).

The results thus demonstrate that although a restriction of the oxygen supply may not contribute to the cellular energy failure *during* hypoglycemia, a reduction of blood flow in the recovery period may well contribute to the final cell damage incurred. It is of interest that regions exist in which the blood flow is reduced to a larger extent than in the fronto-parietal cortex. This finding hints the possibility that some structures may show even less complete metabolic recovery than that previously reported (see Agardh et al 1978). Since the reduction in blood flow was unrelated to arterial hypotension or to hypocapnia and since there is no indication that

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A metabolic basis for the selective vulnerability of neurons in status epilepticus

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Like hypoxia and hypoglycemia, repeated or sustained epileptic seizures can give rise to neuronal damage with a preferential localization to certain cells, mainly small pyramidal cells in the neocortex and hippocampus, and Purkinje cells in the cerebellum. Such a localization has been reported in e.g. bicuculline-induced seizures in spontaneously breathing baboons (Meldrum & Brierley 1973). However, similar studies carried out on paralysed baboons, in which systemic factors such as hypoxia, hypoglycemia, hypotension and hyperthermia could be largely avoided, showed some but less pronounced changes and no cell damage in the cerebellum (Meldrum et al. 1973).

At present, there is no satisfactory explanation for the selective neuronal vulnerability nor has it been possible to define mechanisms that underlie the cell damage. Two findings indicate that the mechanisms are oxidative in nature and not directly related to cellular energy failure. Thus in rats in which status epilepticus is induced by i.v. bicuculline injection cerebral energy state is only moderately perturbed (Chapman et al. 1977) and cell damage seems to be decreased rather than increased if cerebral oxygen supply is curtailed (Blennow et al 1978).

In order to further explore factors that contribute to cell damage in the brain during status epilepticus we have measured local glucose consumption and blood flow. Since the results provide a hint to the mechanisms of damage a preliminary account of the findings is given.

Unstarved, male Wistar rats (290–410 g) were anesthetized with ~1% halothane, tracheotomized, and connected to a respirator delivering 70% N_2O and 30% O_2. Catheters were placed in the femoral arteries and in one femoral vein. Gold-plated screws were inserted in the skull bone for recording of (frontoparietal) EEG. When the operative procedures were completed, halothane supply was discontinued. Arterial P_{CO_2} was adjusted to 35–40 mmHg, and body temperature was maintained close to 37°C.

The animals were allowed a steady state period of about 30 min before seizures were induced by the i.v. injection of bicuculline (1.2 mg kg^{-1} see Chapman et al. 1977). Just before the injection the animals were bled to mean arterial blood pressure of about 120 mmHg to avoid too excessive an increase in pressure at the onset of seizures. Local CMR_{gl} was determined with the method described by Sokoloff et al. (1977). In summary after 20 min of seizure activity 1 ml of ^{14}C-deoxyglucose solution (New England Nuclear), containing 30 µCi, was injected i.v. Over the next 30 min, repeated arterial blood samples were taken for determination of plasma glucose concentration (hexokinase method) and ^{14}C activity (liquid scintillation counting). At the end of this period, the animals were decapitated and the brains were prepared for quantitative autoradiography. Local CMR_{gl} was calculated from the integrated plasma activity and from the ^{14}C activity in tissue. The latter was determined from the X-ray films by microdensitometry either manually (see Abdul-Rahman et al. 1979) or in one case with the automatic microscanning equipment developed in the Laboratory of Cerebral Metabolism, NIMH, Bethesda (courtesy of Dr Louis Sokoloff).

RESULTS AND DISCUSSION

The seizure activity continued unabated before and throughout the period of blood sampling. Typical results on CMR_{gl} are illustrated in the colour photographs obtained with the automatic microscanning equipment (Fig. 1). In frontoparietal cortex (and hippocampus) l-CMR_{gl} exceeded 1.5 µmol g^{-1} min^{-1}. Since this is more than twice the control values (0.6–0.7 µmol g^{-1} min^{-1}) the results show the expected increase in local metabolic rate. More important, though are the large differences in l-CMR_{gl} during seizures. Thus, high values were observed in cortex, hippocampus, thalamus and amygdala and low ones in many other structures including the cerebellum and brain stem. The magnitude of the differences are exemplified by the facts that, during seizures, CMR_{gl} in cortex and hippocampus was about 4 times that measured in cerebellar cortex. Since local differences in CMR_{gl}

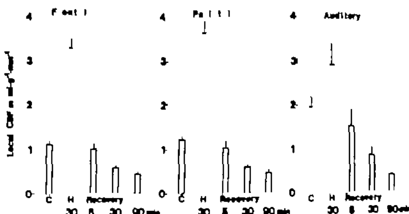


Fig 1 Local cerebral blood flow (*CBF*) in rat cerebral cortex during severe hypoglycemia, and in the recovery period following glucose administration. The values given (means ± S E.) were obtained during control conditions (*C*) after 30 min of hypoglycemia with cessation of spontaneous EEG activity (*H*) and 5 30 and 90 min after glucose administration following a 30 min period of "isoelectric hypoglycemia (*Recovery*) Note hyperemia during hypoglycemia, and progressive reduction in local CBF in the recovery period

the intracranial CSF pressure is appreciably elevated (unpublished results) an increase in cerebrovascular resistance must have occurred In view of its potentially deleterious effect it seems warranted to study whether the increased vascular resistance is du to cell edema or to vasoconstriction and if vasoconstriction is involved to explore the mechanisms whereby it arises

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Fig 1 Local cerebral glucose utilization in the rat brain during bicuculline-induced status epilepticus. The glucose utilization values (μmol per 100 g per min) were obtained by processing the autoradiographic film in the automatic microscanning equipment of the Laboratory of Cerebral Metabolism, NIMH, Bethesda (courtesy of Dr Louis Sokoloff and M C. Goochee). Note high values in the cortex and hippocampus (top panel), and low values in cerebellum (bottom panel).

are much smaller under control conditions (Sokoloff et al 1977 Abdul Rahman et al in preparation) the results emphasize the non-uniform increase in metabolic rate during bicuculline-induced seizures

Under similar conditions of status epilepticus in rats neuronal cell damage was observed in neocortex and hippocampus and to some extent also in thalamus and striatum but lesions were not observed in cerebellum or brain stem (Blennow et al 1978) Obviously there is a striking correlation between local metabolic rate and localization of lesions Unless it can be shown that selectively vulnerable regions show a compromised circulation the results indicate that the development of cell damage is related to an excessive increase in neuronal activity and metabolism

Kerstin Beirup gave excellent technical assistance The authors are deeply indebted to Dr Louis Sokoloff for allowing them to process the autoradiograms in the automatic microscanning equipment The project was supported by funds from The Swedish Medical Research Council (project No B79-14X 263) and the U S Public Health Service via the N I H (grant No 2 R01 NS-07838)

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Presynaptic α-receptors do not depress the secretion of ³H-noradrenaline induced by veratridine

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Blockade of α-receptors very strongly enhances the secretion of ³H-noradrenaline (NA) from the sympathetic nerves of guinea-pig vas deferens evoked by electrical nerve stimulation, but not that caused by direct depolarization of the nerve terminals with high potassium (Stjärne 1978 Stjärne Alberts & Bartfai 1979). While the electrically evoked secretion is blocked by tetrodotoxin and thus requires intact impulse propagation in terminals, that caused by high potassium is resistant to this toxin, and thus due to direct depolarization of varicosities. The results suggest that α-receptor mediated presynaptic inhibition of NA secretion ('α-autoinhibition') does not to any major extent depress depolarization-secretion coupling in varicosities but may rather operate by restricting invasion of nerve endings by nerve impulses, thus preventing activation of the more distally located varicosities of each branch. The mechanism whereby stimulation of presynaptic α-receptors by NA secreted from an activated varicosity could cause such intraterminal conduction block may be promotion of potassium efflux from the nearby parts of the terminal leading to highly localized hyperpolarization of the nerve membrane. This would lower excitability and therefore the probability that the nerve impulse will reach the firing level in the next thin intervaricose segment. The primary target of α-autoinhibition would in that case be the potassium conductance of the terminal membrane rather than its calcium conductance, as commonly assumed (Stjärne 1978, Stjärne et al. 1979).

If the hypothesis is correct, blockade of 'α-autoinhibition' should much more strongly increase the secretion of ³H-NA evoked by electrical nerve stimulation than that caused by veratridine, an agent shown to cause exocytotic secretion of NA from adrenergic nerves, including those of guinea-pig vas deferens (Thoa et al. 1975) by a direct action on varicosities (probably by blocking the inactivation of their sodium conductance (Ohta, Narahashi & Keeler 1973)).

Guinea-pig isolated vas deferens, prelabelled with ³H-(−)-NA and superfused with Tyrode solution (calcium 1 8 mM) was used for the study (the methods have been described in detail elsewhere Stjärne 1977). Desipramine (0 6 μM) and normetanephrine (10 μM) as well as atropine (2 6 μM) were present in the medium to prevent rebinding of NA and to prevent cholinergic interference. The fractional, stimulus-induced (transmural electrical nerve stimulation, or addition of veratridine) rise in efflux of ³H (essentially ³H-NA) was used to estimate the secretion of ³H NA (Δt) which is defined thus,

$$\Delta t = \frac{\text{Evoked rise in } ^3\text{H efflux}}{\text{Total } ^3\text{H in the tissue at the time of stimulation}}$$

The secretory response to veratridine was quite variable in the vasa deferentia from the different animals used and also showed considerable tachyphylaxis. Because of this a fixed protocol was adopted, and in each experiment one vas deferens was used as control (intact 'α-autoinhibition') while the contralateral vas was 'experimental' ('α-autoinhibition' blocked with phentolamine 1 μM). As shown in Fig. 1 exposure to veratridine (12.5 25 and 50 μM added in that sequence for a total of 4 min) caused a dose dependent rise in Δt (fractional secretion of ³H NA per stimulation period) both in the absence and in the presence of phentolamine. However while removal of α-autoinhibition (with phentolamine) caused a highly significant rise in the secretory response to indirect activation of varicosities by electrical nerve stimulation (by a factor of 3.37±0.21 $P<0.001$) it did not significantly alter the response to direct secretory activation of

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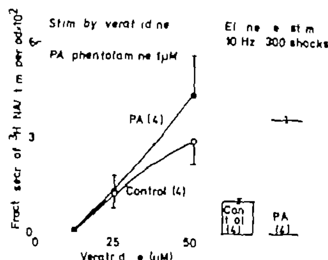


Fig 1 Effects of phentolamine on the fractional secretion of H NA per stimulation period evoked by veratridine and by electrical nerve stimulation Means ± S E ($n=4$)

varicosities with veratridine (ratio of Δr in the presence of phentolamine to Δr in controls throughout the veratridine dose range 1 28×0 22 $P>0\ 05$)

Taken together with the above mentioned finding *that removal of α autoinhibition does not markedly* enhance the secretion evoked by direct depolariza tion of varicosities with high potassium the present results provide strong evidence that the target of α autoinhibition is not or not to any major extent *depolarization secretion coupling in individual var* icosities This adds further weight to the hypothesis that α-autoinhibition of NA secretion may rather *be exerted by depression of intraterminal conduc* tion of nerve impulses and therefore be an expression of failure of nerve impulses travelling in the nerve trunk *to invade and recruit the more distal*ly located varicosities in the terminal branches of th nerve (Stjärne 1978 Stjärne et al 1979) The evi dence for this is still indirect however Speci electrophysiological techniques are required *conclusively demonstrate whether or not the hy*pothesis is correct

This work was supported by grants from the Swedi Medical Research Council (project B79-04X-03027-10B from Magnus Bergvalls Stiftelse and from Karolinska In stitutets Fonder I thank Mrs Eivor Stjärne and M Ingmarie Eriksson for excellent technical assistance.

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Release of adenosine from the rabbit heart by sympathetic nerve stimulation

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There is considerable evidence that adenosine plays a role as a physiological regulator of coronary flow as originally proposed by Berne (1963). In addition adenosine inhibits the increase in heart rate ventricular pressure and *dp/dt* caused by catecholamines (Schrader et al. 1977) as well as the metabolic actions of these agents in the heart (Dobson 1978). Finally we recently showed that adenosine inhibits the release of noradrenaline from the perfused rabbit heart (Hedqvist & Fredholm 1979). The present communication presents evidence that adenosine is released by sympathetic nerve stimulation in the same preparation, and thus suggests that the purine may act as a pre and post junctional modulator of adrenergic neurotransmission, in addition to being a coronary vasodilator

Rabbits of either sex, weighing 1.5–2.5 kg, were killed by a blow on the head. After exsanguination the heart with its sympathetic nervous supply was dissected out and perfused according to the Langendorff technique with Tyrode s solution (see Hedqvist & Fredholm 1979). Perfusate was collected before, during and after sympathetic nerve stimulation (10 Hz, 1 ms duration 20V) and immediately frozen for subsequent assay of adenosine, inosine and hypoxanthine by high performance liquid chromatography as described in detail elsewhere (Fredholm & Sollevi 1979). Aliquots (0.1 ml) of the effluents were chromatographed on a μ-Bondapack C 18 column using 0 01 M $NH_4H_2PO_4$ pH 6.0, containing 15% methanol, as the mobile phase. The compounds were identified by their retention times (hypoxanthine 4 7 min, inosine 5 6 min, adenosine 11.5 min at a flow rate of 1.8 ml/min, see Fig. 1) and quantified by the peak absorbance at 254 nm. The detection limit was 1–5 pmol for either substance

Adenosine and inosine was identified in extracts of freeze clamped rabbit hearts purified by phenylboronate affinity chromatography according to Fredholm & Sollevi (1979) In non-stimulated heart the adenosine level averaged 2 4 nmol/g and in heart stimulated with 10 Hz it averaged 10 4 nmol/g ($n=3$) This value for adenosine in resting heart is similar to that found in heart from other species (see Arch & Newsholme 1979) The outflow of inosine and adenosine increased in parallel with the tissue level Thus, the 10-fold increase in tissue level in these experiments was accompanied by a 10-fold increase in perfusate adenosine concentration (from 24 to 196 nmol/l) and a 5-fold increase in inosine concentration (from 93 to 431 nmol/l) Typical chromatograms of heart perfusates are shown in Fig. 1 It clearly shows that the peaks corresponding to inosine and adenosine increase markedly in height during and following nerve stimulation with 10 Hz. Other (unidentified) peaks were essentially

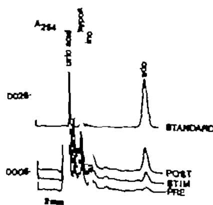


Fig 1 Tracing from original chromatograms of perfusates of rabbit heart. 100 μl perfusate was administered at the sharp deflection in the far left of the tracing The lowest tracing is a perfusate from resting heart, the second one obtained during 60 nerve stimulation at 10 Hz, the third from below perfusate taken 60 after the same stimulation. The uppermost tracing shows the chromatogram of standard solution containing uric acid hypoxanthine, inosine and adenosine (50 pmol of each)

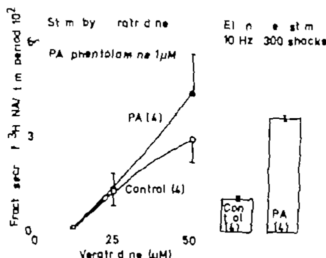


Fig. 1. Effects of phentolamine on the fractional secretion of ³H NA per stimulation period evoked by veratridine and by electrical nerve stimulation. Means ± S.E. ($n=4$).

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...a Physiol Scand 1979 106, 383–384

...asma norepinephrine concentration during submaximal and ...ximal exercise at lowered skin and core temperatures

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Key words Hypothermia, submaximal and maximal exercise, norepinephrine heart rate, oxygen uptake, blood pressure

Oxygen uptake and heart rate during maximal exercise are reduced during hypothermia. Heart rate during submaximal exercise is reduced as well (cf ...bbl et al 1974). The mechanisms for these changes during hypothermia are unknown, but one possible reason is changes in the autonomic nervous system. However administration of atropine did not reverse the bradycardia, which was observed during maximal exercise in hypothermia (Davies et al. 1975). The question then arises, whether or not the β-receptor activity is reduced in this situation.

Exercise in cold water increases concentrations of plasma norepinephrine over values observed in ... water (cf Johnsson et al 1977) However the effect of lowering of core temperature has not been studied in a way that would separate core and surface temperature effects. Therefore 2 moderately trained men 39 and 42 years old performed submaximal bicycle and maximal simultaneous arm and leg exercise in the following 4 experimental situations.

1) Normal core—normal skin temperatures (NN)
2) Lowered core—normal skin temperatures (LN)
3) Normal core—lowered skin temperatures (NL)
4) Lowered core—lowered skin temperatures (LL)

Core temperatures were reduced by non-exercising immersion in cold water (13–15°C) Reduced skin temperature was obtained by cooling the laboratory constantly moving the air with fans and continuously spraying subjects with cold water. Temperatures were measured with thermocouples: core temperatures (T_{oe}) continuously with a thermocouple in the esophagus at the level of the heart mean skin temperatures ($\bar{T}_{sk}$) at 6 different skin locations, and vastus lateralis muscle temperature (T_m) immediately after the exercise period with a needle containing a thermocouple Plasma concentrations of norepinephrine (NE) were measured by the method described by Lake et al. (1978)

Control values of HR, blood pressures (cuff method) and vein blood NE were first obtained after 15 min of supine rest. Thereafter in the experimental situations with low T_{oe} the subjects stayed in the cold water until the T_{oe} was approximately 35°C. On the other two experimental days the subjects went directly to the testing laboratory In the laboratory the different values were again obtained after 10 min of rest sitting on the bicycle and during or after the submaximal and maximal exercise

Preexperimental control values for HR, BP and NE were almost the same on the 4 different experimental days Oxygen uptake and HR varied as expected and previously described (Table 1) At rest and during submaximal exercise V_{O_2} was increased in the three situations with low $\bar{T}_{sk}$ and/or low T_{oe}. However V_{O_2} was increased most when both T_{oe} and $\bar{T}_{sk}$ were low During maximal exercise V_{O_2} was reduced with both low core situations (LN and LL) but the reduction was not observed with lowered skin temperatures alone

Resting values of NE were increased in all low temperature experiments (Table 1) In fact the elevation with resting LL was almost as much as maximal exercise in NN This pattern of higher values for LN and NL than NN and the highest values for LL was also present in both submaximal and maximal exercise.

Blood pressure values increased during exercise

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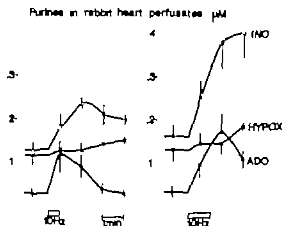


Fig 2 Concentration of adenosine (●—●) inosine (○—○) and hypoxanthine (×—×) in perfusates from rabbit hearts before *during* and after stimulation of the sympathetic nerves (10 Hz, 1 ms 20V) for 30 s (left part, $n=6$) or 60 s (right part $n=3$) Mean ± S E

unchanged The compiled results on purine release from 9 expts (separate from those in which tissue levels were determined) are summarized in Fig 2. Following stimulation of the nervous supply with 10 Hz there is a rapid increase in perfusate adenosine levels The concentration of inosine the first breakdown product increased somewhat later and the level of hypoxanthine the metabolite of inosine increased still later after stimulation It is interesting to note that stimulation for 1 min caused a considerably larger increase of both adenosine (6-fold vs 3 fold) and inosine (2.5-fold vs 2 fold) than stimulation for ½ min The perfusate flow rate averaged 27 ml/min Therefore the basal rate of adenosine and its breakdown products corresponds to 8 nmol/min On a weight basis the present value (1.5 nmol min g [1]) is similar to that found in the perfused rat heart but higher than that found in guinea pig cat and dog (see Arch & Newsholme 1979) The peak rate of efflux after stimulation 10 Hz for 1 min was 19 nmol/min We have shown previously (Hedqvist & Fredholm 1979) that the addition of adenosine to the perfusate to a final concentration of 1 μmol/l reduces the overflow of noradrenaline induced by 10 Hz nerve stimulation by close to 40% This corresponds to a rate of administration of 20–30 nmol/min i e very close to the amounts formed endogenously by the same stimulation

When adenosine was added in a concentration of 2 5 μmol/l in the perfusate only 0 65 μmol/l was recovered in the effluent. The sum of adenosine inosine and hypoxanthine was 1.2 μmol/l indicating that more than half of the adenosine is inactivated by uptake in a single passage through th Langendorff perfused heart.

The inactivation of adenosine by uptake an metabolism is unlikely to be lower when th adenosine is produced close to the site of inactiva tion than when the adenosine is given via the perfu sate These findings further underline the similarit between the amounts of adenosine produced an the amounts found to exert pronounced cardiac ef fects Furthermore the tissue levels of adenosin found (1–23 nmol/g) corresponds to concentratio in the tissue of more than 1 μM i e a level that ha pronounced effects

In conclusion the present results show that sym pathetic nerve stimulation leads to the formatio and release of adenosine in the rabbit heart Th amounts formed appear to be sufficient to produc a number of effects including inhibition of nor adrenaline release. The data are thus in agreemen with the view that adenosine could act as a physiologically important transsynaptic modulato of noradrenaline release (Fredholm & Hedqvist 1979 Hedqvist & Fredholm 1979)

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..enal cortical blood flow distribution measured ..y hydrogen clearance during dopamine and ..cetylcholine infusion Effect of ..lectrode thickness and position in cortex

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TYSSEBOTN I. & KIRKEBØ A Renal cortical blood flow distribution measured by hydrogen clearance during dopamine and acetylcholine infusion. Effect of electrode thickness and position in cortex. Acta Physiol Scand 1979 106. 385–393 Received 16 Nov 1979 ISSN 0001–6772. Institute of Physiology University of Bergen, Norway

Blood flow distribution in the renal cortex was investigated in control and during i.a. infusion of dopamine (DA) and acetylcholine (Ach) in dogs. Local blood flow in outer cortex (OCF) and in inner cortex (ICF) was measured by platinum electrodes detecting hydrogen washout rate in tissue. Mean cortical blood flow measured by hydrogen washout rate in the renal vein (CFV) was compared with renal arterial blood flow (RAF) measured by electromagnetic flowmeter With electrodes of 0.05–0.2 mm diameter control blood flow rates in outer and inner cortex were 4.57± (S D) 1 73 ml/min g and 4.35±0.57 ml/min g, which is higher than found using 0.–0 5 mm electrodes in this and previous studies. OCF and ICF increased proportionally during intraarterial infusion of DA or Ach. The increase in local blood flow per unit volume was about 20% less than the increase in RAF most likely due to an increase in renal volume and reduced vasodilatory response in the surrounding of some electrodes. CFV rose almost to the same degree as RAF showing diffusion equilibrium for hydrogen gas even at maximal flow rate During vasoconstriction induced by high doses of DA, OCF and ICF fell proportionately Thus, equal vascular responses in outer and inner cortex were observed during both vasodilator and vasoconstrictor infusion. This indicates that changes in sodium excretion with renal blood flow may not be associated with a redistribution of cortical peritubular blood flow

Key words. Renal blood flow distribution, hydrogen clearance, acetylcholine dopamine

Dopamine (DA) is found in high concentration in kidneys of several species (Goldberg 1972) and Cuche et al. (1972) have suggested that DA released within the kidney participates in the regulation of sodium excretion and local blood flow

The increased renal blood flow induced by vasodilating agents is usually accompanied by a rise in sodium excretion with no or only a small elevation of glomerular filtration rate (Pinter et al. 1964) Based on ⁸⁵Kr data Barger (1966) suggested a redistribution of renal blood flow and glomerular filtration to outer cortex and superficial saltloosing nephrons in models associated with natriuresis. Aukland & Loyning (1970) found only a 6% rise in medullary blood flow measured by H clearance whereas total renal blood flow increased 54% during acetylcholine (Ach) infusion They suggested that juxtamedullary arterioles are less responsive than other cortical arterioles to such vasodilator action

On the other hand results obtained with microspheres showed increased fractional blood flow to inner cortical zones during infusion of Ach (McNay & Abe 1970, Stein et al. 1971) or of DA (Hardaker & Wechsler 1973) compatible with the theory of Earley & Friedler (1965) who assumed that an increase in inner cortical and medullary blood flow would reduce sodium reabsorption However Clausen et al (1978) could confirm an increased microsphere fraction in inner cortex during vasodi-

Table 1 *The means of physiological responses to the cold and exercise of two subjects*

	Rest				Submaximal exercise				Maximal exercise			
	NN	LN	NL	LL	NN	LN	NL	LL	NN	LN	NL	LL
T_{es} °C	36.5	35 0	36 8	34.8	37 0	35 7	36.5	34 9	37.8	35 9	36.5	35.2
T_m °C	34.3	31.5	31 9	32.5	37.3	34 7	36.1	35 1	38 7	35.6	36.8	35 5
T_{sk} °C	31 4	30.3	21.8	1 8	31 6	30 9	20 7	20 6	31.5	31.5	20 4	21.3
HR, bpm	63	49	66	74	100	87	96	101	167	151	157	151
V_{O_2} l min	0 35	0 51	0.59	1 22	1.57	1 88	1.86	2.18	3 85	3.38	3.80	3 4[illegible]
La mM	1 1	2 6	1 4	5.8	2.6	3 0	2.0	5 6	9 4	10.3	7 4	11.2
aBP mmHg	124/86	135/94	136/91	137/85	149/78	137/76	150/77	150/88	190/80	167/69	171/75	173/[illegible]
Ne pg ml⁻ 10⁻⁴	0 8	1 8	1 9	4 1	1.3	2 0	3 1	6 2	4 4	5.5	8 7	10.[illegible]

but no clear pattern of response to the cold could be observed The BP appeared to be somewhat lower during maximal exercise in LL than in the NN situation BP values do not follow the same pattern as NE and had no relation to NE

In summary The plasma norepinephrine concentration was highest in the LL experimental situation and lowest in the NN situation Intermediate values were noted in the LN and the NL situations These results strongly suggest that both cold skin and cold core activate the sympathetic nervous system and it further suggests that these effects may be additive These findings indicate that the reduced HR during exercise in hypothermia cannot be explained by diminished sympathetic activity However the possibility does exist that beta receptor activity is reduced in hypothermia or that there is diminished responsiveness to endogenous norepinephrine Furthermore the cold induced increase of NE at rest and during exercise did no[t] result in an elevated BP

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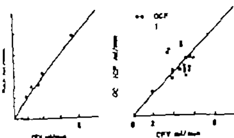


Fig 1 Renal arterial blood flow (*RAF*) and local blood flow in outer cortex (*OCF*) or inner cortex (*ICF*) related to cortical blood flow (*CFV*) measured by electrode in the renal vein in control (filled symbols) or during acetylcholine infusion (open symbols).

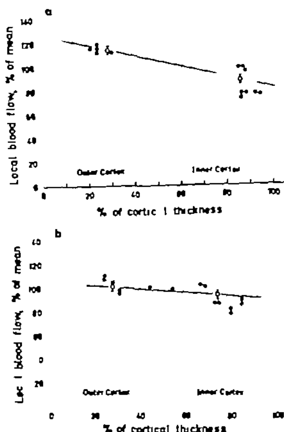


Fig 2 Blood flow at single electrode sites in per cent of mean local flow in each expt. related to the relative depth of the electrode tips in cortex, 100% cortical depth refers to the corticomedullary border. Open symbols give the verage blood flow (± S E.) and average electrode position in each zone. ( ) Control flow measured with 0.5 mm electrodes (Group II) (*b*) Control flow measured with 0.2 mm electrodes (Group III)

RESULTS

Cortical blood flow distribution measured by 0.5 or 0.2 mm electrodes

Control measurements were performed after a recovery period of 60 min after completing surgery. The mean of 2 measurements made within 10 min before starting infusion of vasoactive agents was used as control value for each infusion period. A verage arterial blood pressure (AP) for each of the three groups was 122 or 123 mmHg.

In group I, DA infusion, the average control of renal arterial blood flow (RAF) was 4 48±1.39 ml/min g (mean ± S.D) Average outer cortical blood flow (OCF) was 3 79±0 98 ml/min g and average inner cortical blood flow (ICF) 3 02±0.65 ml/min g measured by the 0.5 mm electrodes (Fig 3)

In group II in which the 0 5 mm electrodes were used, average RAF was 4 70±1 47 ml/min g before vasodilation by infusion of Ach in the renal artery whereas OCF averaged 4 09±0.83 and ICF 3 30±0 66 ml/min g (Fig 4)

When the 0 mm electrodes were used (group III), higher local blood flow was obtained than with 0.5 mm electrodes Control OCF for this group was 4 97 1 73 ml/min g and ICF was 4 35±0 57 ml/min g (Fig 5), the latter significantly h gher (P<0 01) than ICF measured by 0 5 mm electrodes. Average RAF 4 09±1.31 ml/min g, however was slightly lower than in the two other groups but agreed well with local cortical blood flow and with renal cortical blood flow (CFV) 4 19±1.27 ml/min g measured simultaneously in the same dogs by platinum electrodes in the renal vein A close correlation between CFV and RAF was found for the whole flow range observed whereas the relation between CFV and local blood flow showed considerable scatter (Fig 1) This indicates that the higher control local flow values obtained by 0.2 mm electrodes than by 0.5 mm electrodes may be due to the electrode type and do not reflect real higher cortical blood flow in these dogs.

To test whether 0 1 mm electrodes with cone tip diameter 0 02–0.1 mm would further raise the control local blood flow three such electrodes and three 0.2 mm electrodes were inserted midcortically in 4 kidneys Less than 2% deviation in average local flow was detected from 96 washout curves (48 positions) recorded simultaneously with the types of electrodes

lation but they did not observe any redistribution of iodoantipyrine or tritiated water uptake in the same kidney

Because of these conflicting results obtained with different flow methods during renal vasodilation we found it desirable to reinvestigate the effect of Ach and DA on cortical blood flow distribution using H_2 washout technique (Aukland et al 1973) This method allows repeatable measurements of local peritubular capillary blood flow However it has not previously been documented that the H_2 method is capable of measuring high cortical blood flow Since we used a new thinner type of platinum electrode and obtained higher control blood flow in dog than previously reported with this method we also compared local H_2 washout rates with the washout rate measured in the renal vein and with RAF

METHODS

The effect of Ach and DA on renal blood flow was determined in 30 expts on 24 dogs weighing 13–25 kg The dogs had free access to food and water until 12 h prior to anesthesia induced by i v infusion of Nembutal 25 mg/kg b wt Additional doses of Nembutal were given as needed to maintain an adequate anesthetic level All dogs ventilated spontaneously through an endotracheal rubber cannula of suitable dimension

Polyethylene catheters were placed in the saphenous artery and vein for arterial pressure recording and infusion The kidney was exposed retroperitoneally through a flank incision and gently dissected free A polyvinyl catheter was sewn into the renal artery with the tip located upstream This catheter was cut 3–4 cm from the renal artery and connected to two PP90 catheters through a y-cannula one catheter leading to a syringe pump (Sage instrument Mod 355) for infusion of Ach or DA dissolved in 0 9% saline while the other was used for injections of H_2-saturated saline The kidney was denervated in all expts. by excision of all visible nerve fibres to the kidney In 6 of the expts a Blalock clamp was placed on the aorta above the renal artery for graded reduction of renal flow

L-shaped platinum electrodes were inserted into the cortex at different depths 2 or 3 with the sensitive tip located in the outer half and 2 or 3 in the inner half of cortex The shafts of the electrodes were fi ed to the renal capsule by sutures and the electrodes carefully covered with perirenal fat In 7 expts a platinum electrode mounted in a catheter was introduced into the renal vein from a femoral vein (Group III) The sensitive platinum tip protruding 2–3 mm from the end of the catheter was tentatively located midstream in the renal vein In these expts the animals were heparinized by 5 000 IU Heparin to prevent clotting around the venous electrode

The H_2 concentration around the sensitive electrode tips was determined polarographically at a polarization potential of +0.2V against an Ag/AgCl electrode placed subcutaneously on the hip (Aukland et al. 1973) and the H current recorded on a 6-channel recorder (Rikadenki Kogyo Co Model B-64) H_2-saturated saline at 37°C injected by hand until a constant H current was obtai from the different electrodes, the injection was then s denly stopped and the washout curves recorded Th curves were generally monoexponential down to ca. 1(of initial saturation The halflife of H in the tissue (was determined from a semilogarithmic plot of the wa out curves and local blood flow calculated from the f mula $F/V = \lambda \ln 0.5/T_{\frac{1}{2}}$ ml/min g.

The total renal arterial blood flow (RAF) was det mined by an electromagnetic flowmeter directly c brated on a femoral artery

After each expt the kidney was excised drained fo min and weighed for calculation of RAF in ml/min g. T position of each electrode tip was carefully examined a its relative depth in the renal cortex was calculated. T animals were divided into 3 groups.

Group I Dopamine infusion

The effect of DA on the cortical blood flow was tested 10 expts using Pt-electrodes with a total diameter of (mm and a sensitive cone tip of 0. –0.5 mm thickne Increasing doses of DA from 10 µg/min (0.1 ml/min) a upward were employed until maximal vasodilation w recorded by the electromagnetic flowmeter The loc flow was measured at medium and maximal dilatation.

When the DA dose was increased to about 700 µg/m the RAF fell to low levels and the local cortical washo rate was determined at a stable level of RAF Infusion were repeated with intervals providing recovery of RA to control level between each infusion

Group II Acetylcholine infusion
0.5 mm electrodes

The effect of Ach on the renal cortical blood flow wa tested in 13 expts. using Pt-electrodes with a diameter o 0.5 mm Varying doses of Ach (2.5–25 µg/min (0.1 ml/min) were tested until maximal vasodilatation was ob tained Local flow was determined at each level after a stable RAF had been obtained usually after 2 5 min

The renal arterial pressure was then stepwise lowered unto 50–60 mmHg by constricting the aorta using the Blalock clamp and local and total renal blood flow measurements were repeated

Group III Acetylcholine infusion
0 2 mm electrodes in cortex and renal vein

In 7 expts. a thinner type of teflon insulated Pt-electrode was used The total diameter was 0.4 mm and the bare tapered electrode tip had a length of 0 5 mm and diameter 0.05–0.16 mm

Five electrodes were placed in the cortex and 1 electrode in the renal vein as described above and H washout rate was recorded simultaneously with electromagnetic flow registration at different doses of Ach

Test of 0 1 mm electrod

In 4 kidneys three 0.4 mm electrodes and three 0.1 mm electrodes were inserted midcortically and local H washout rates recorded simultaneously in control condition.

Statistical significance was assessed with Student's t tests.

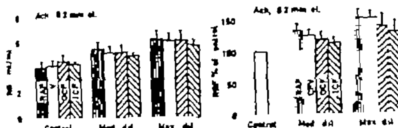


Fig. 5. Average renal blood flow (*RBF*) (± S.E.) in control and during infusion of acetylcholine causing medium or maximum vasodilation, Group III. *OCF ICF* outer and inner local cortical blood flow were measured using 0.2 mm electrodes. *RAF* renal arterial blood flow. *CFV* cortical blood flow measured by electrode in the renal vein.

2.0+1.22 ml/min g (48%) and local blood flow to the same degree (Fig. 3): OCF 1.80±1.15 ml/min g (67%) and ICF 1.31±0.58 ml/min g (46%) were equally reduced. Thus the responses to DA were similar in outer and inner cortical zones both for vasodilator and for vasoconstrictor doses.

Acetylcholine infusion, 0.5 mm electrode (Group II). RAF stabilized at a higher level within 2 min after start of Ach infusion. We were not able to induce renal vasoconstriction by Ach even when the Ach doses infused in the renal artery lowered heart rate and AP. However with continued infusion of high doses of Ach an escape of the vasodilator response was detected and the dose had to be increased to keep RAF constant.

RAF increased maximally to an average of 7.5 ... ml/min g (160% of the control value) (Fig. 4). The corresponding local cortical flow increased less than RAF. OCF rose to 5.34±0.90 (105) and ICF to 4.34±0.67 ml/min g (140%) indicating a similar increase in local blood flow in both cortical halves during Ach infusion. The results obtained at medium dilation were principally identical (Fig. 4).

Aortic constriction during maximal vasodilatory infusion of Ach reduced RAF to 2.09±1.17 ml/min g (44%). OCF and ICF fell to 1.79±0.77 ml/min (46%) and 1.65±0.50 ml/min g (47%) respectively demonstrating a close correlation between changes in OCF ICF and RAF when renal blood flow was reduced (Fig. 4).

Acetylcholine infusion, 0.2 mm electrodes in cortex and renal vein (Group III). In group III using 0.2 mm electrodes, the relative increase in total and local renal blood flow was in general accordance with the results obtained with 0.5 mm electrodes, but the absolute local cortical flow agreed far better with total renal flow measurements. At maximal vasodilation by Ach RAF rose to 6.22±1.87 ml/min g (153%) slightly less than in the second group but close to the rise in CFV averaging 6.09±1.60 ml/min g (151%) measured by H electrodes in the renal vein (Fig. 5). The increase of OCF to 6.11±2.18 ml/min g (137%) and of ICF to 5.68±1.18 ml/min g (130%) was about 15–20% less than the corresponding increase in RAF and CFV but the absolute blood flow values showed close agreement during both maximal and moderate dilation. The ratio OCF/ICF was unchanged during Ach infusion, as with 0.5 mm electrodes.

Also with the 0.2 mm electrodes local flow measurements at single electrode sites showed considerable scatter. At the majority of sites a proportional or slightly smaller rise in local flow than in RAF was recorded during Ach infusions. A few electrode sites even showed greater increase in local than in total renal blood flow. However some electrode sites, 1–2 of 6, showed a small rise in flow, thus lowering average local blood flow. RAF is not corrected for volume expansion during vasodilation and therefore overestimates flow changes per unit tissue weight during vasodilation.

The effect of Ach on local cortical flow seemed to be delayed compared to the increase of RAF. Therefore 3 expts. were made to test the time course of vasodilation on different electrode sites compared to RAF which increased to an almost stable level within min. The results from one typical dog are plotted in Fig. 6 clearly showing that a progressive increase in local flow occurred in the first 10 min after the start of Ach infusion. After 10 min no further increase was observed. In some cases of moderate vasodilation the measurement of local flow was made in the critical time period, 5–10

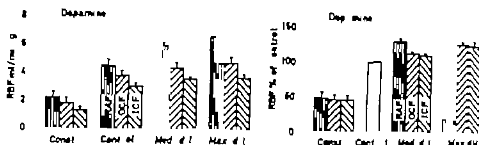


Fig 3 Average renal blood flow (*RBF*) (± S E) in control and during infusion of 3 different doses of dopamine causing medium and maximal vasodilation or vasoconstriction Group I *RAF* renal arterial blood flow *OCF* outer cortical blood flow *ICF* inner cortical blood flow

The local cortical flow rates depend also on the depth of the electrode tips in cortex In Fig 2*a b* the flow rate at single electrode sites relative to mean local flow in each expt is plotted against the relative depth of the single electrode tips in cortex (100% cortical depth refers to the cortico-medullary border) Fig 2*a* refers to the series of expts with 0 5 mm electrodes and shows distinctly higher local flow rates in outer cortex than in inner cortex Average OCF and ICF were 113% and 88% of mean local flow giving a ratio OCF/ICF of 1 29

Fig 2*b* presents the corresponding values obtained by single 0 2 mm electrodes OCF was 103% and ICF 96% of mean local flow showing a smaller ratio OCF/ICF of 1 06 for this series The average depths of the electrodes in outer cortex were identical 29% for both series Average depths of electrodes in inner cortex however were 74% and 86% respectively for the series using 0 2 and 0 5 mm electrodes Thus the observed discrepancy in OCF/ICF ratio between the two series can be explained partly by the deeper H_2-sampling sites in inner cortex when 0 5 mm electrodes were used causing low ICF in these series and thereby a higher OCF/ICF ratio

Dopamine infusion (Group I) Infusion of moderate doses of DA in the renal artery increased renal blood flow in all kidneys whereas high doses caused renal vasoconstriction At the dose inducing maximal vasodilation RAF rose to 6 65±1 48 ml/min g (153% of the control value) (Fig 3) Local blood flow measured simultaneously by the H_2 desaturation rate increased only half as much as RAF OCF rose to 4 75±1 33 ml/min g (127%) which is similar to the rise in ICF 3 71±0 70 ml/min g (125%) showing equal vasodilation in both cortical zones Principally similar results were also obtained with lower doses of DA (Fig 3) The vasodilatory response at single electrode sites showed considerable scatter the flow increase was less than 10% at some sampling sites while at a few other sites the relative rise in flow was higher than the rise in RAF

When the DA infusion was increased a vasoconstrictor response was observed and the renal blood flow could easily be completely stopped. DA doses causing low stable total flow reduced RAF to

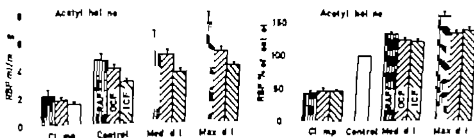


Fig 4 Average renal blood flow (*RBF*) (± S E) in control and during infusion of acetylcholine causing medium or maximum vasodilation Group II "Clamp" refers to results obtained by aortic clamping reducing arterial perfusion pressure during maximal vasodilation. *OCF ICF* outer and inner cortical blood flow were measured using 0.5 mm electrodes *RAF* renal arterial blood flow

[illegible] in outer cortex but differs in inner cor[illegible], as in the present study cortical blood flow is [illegible] uniform, indicating a discrepancy between [illegible] measurements based on glomerular blood flow [illegible] on peritubular flow.

Labelled microspheres trapped in the glomerular [illegible]ry bed are known to be reliable for measure[illegible] of the total renal blood flow whereas it is still [illegible] [illegible]sion whether microsphere distribution [illegible] true zonal glomerular blood flow. Trapping [illegible] microspheres may depend also on plasma skim[illegible] and steric restriction. The distribution of large [illegible] is different from the distribution of smaller [illegible] in cortex (Marlzrid et al. 1976, Ishikawa & [illegible]berg 1977) and we suspect that microspheres [illegible] underestimate deep cortical blood flow in con[illegible].

[illegible]duced renal blood flow

[illegible] of DA in large doses resulted in a pro[illegible] fall in RAF and a proportional reduction of OCF and ICF. These observations agree well with [illegible] findings using the H technique (Tys[illegible] & Kirkebø 1975) and recently by the [illegible] antipyrine method (Clausen et al. 1978) during infu[illegible] of vasoconstrictor agents. A similar flow re[illegible] in all cortical layers has also been obtained by microspheres (Rector et al. 1972) whereas Clausen et al. (1978) found relatively fewer spheres distributed to inner cortex *during* i.a. infusion of large doses of angiotensin II.

As the vasoconstrictor effect of DA is mediated by α-adrenergic receptors (see Goldberg 1972) and noradrenalin infusion causes homogeneous reduc[illegible] of cortical flow measured by H washout technique (Tyssebotn & Kirkebø 1975) a parallel response might have been anticipated for DA.

Renal blood flow was reduced also by aortic con[illegible] proximal to the kidney maximally vasodi[illegible] by Ach. This procedure allowed measure[illegible] of flow distribution and vasodilatory capacity [illegible] low flow rates at which measurements with the [illegible] method previously are shown to be compatible with the total renal blood flow measurements (Auk[illegible] et al. 1973).

As RAF and local flow decreased to the same [illegible] and the cortical flow distribution was un[illegible] at these low flow rates, the evidence for [illegible] [illegible]dilatory capacity in outer cortex and in[illegible] cortex also at high flow rates was supported.

Increased renal blood flow by DA or Ach infusion

Infusion of DA and Ach increased total renal blood flow maximally to average 150–160% of control values. OCF increased in the same proportion as ICF. This result stands in contrast to observations made with microspheres. Hardaker & Wechsler (1973) found that DA infusion caused a relative redistribution of microspheres from the outer to the inner renal cortex. Similar results were obtained by McNay & Abe (1970), Stein et al. (1971) and Clausen et al. (1978) during Ach administration and by Baehler et al. (1973) using frog red cells as well as microspheres. On the other hand, in accordance with the present results, Clausen et al. (1978) detected no changes in flow distribution during Ach vasodilation measured by I-antipyrine and tritiated water. They concluded that "the disparity between microspheres and diffusible tracers indicates a disability of microspheres to enter the inner cortical arterioles in the same proportion as blood flow. A second possibility would be a variable postglomerular capillary flow component with inward direction in cortex.

A third possibility is that during the washout period H_2 gas from the inner cortex diffuses into the interlobular arteries and is transported to outer cortex. Thereby OCF will be underestimated.

Measurements with platinum electrodes in cortex showed a 15–20% lesser increase in local flow during dilatation than the increase in total renal blood flow. This discrepancy was in part due to differences in electrode response at single electrode sites. The recorded increase in washout rate on most electrodes was slightly less or of the same extent as the rise in RAF. Some electrodes, 1–2 out of 6, recorded much lower increases in washout rate during vasodilation.

However, CFV measured by a platinum electrode in the renal vein was closely correlated with RAF measured by electromagnetic flowmeter. This clearly indicated that H equilibration in cortex was not a limiting factor at any flow rate determined by the venous electrode. Thus, the restrained response to vasodilation at some single electrode sites represents a local phenomenon in the small volume around the electrode and is not explained by a general diffusion limitation of H_2 washout. Insertion of some of the electrodes may have caused bleeding, edema formation or impaired vascular reactivity

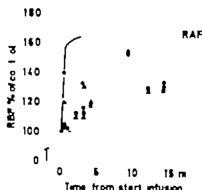


Fig 6 Increase of renal arterial blood flow (RAF) compared to changes in blood flow at single electrode sites in outer (closed symbols) and inner (open symbols) cortex after the beginning of infusion of acetylcholine in one expt. RBF renal blood flow

min after start of Ach infusion and consequently might partly explain why the rise in local flow was smaller than total flow elevation

DISCUSSION

Control local blood flow measured by 0.5 and 0 2 mm electrodes

The control local cortical blood flow measured by 0 5 mm platinum electrodes as well as electromagnetically measured RAF corresponds well to results obtained in previous studies performed with the same technique (Loyning 1971 Aukland et al 1973 Tyssebotn & Kirkebo 1975 1977) Local cortical flow measured by 0 2 mm electrodes was compatible with total renal blood flow determined by arterial flow probe or by venous electrode On the other hand electrodes of 0 2 mm diameter measured ICF significantly higher than earlier described ($P<0.01$) and close to the flow in outer cortex which was not significantly higher than flow measured with 0.5 mm electrodes If the difference is due to damage to the cortical tissue caused by the insertion of the electrode the damage would most likely be less with thinner electrodes and this would be one likely reason for the higher cortical flow measured with 0 2 than 0.5 mm electrode diameter However further reduction of the electrode diameter did not alter the average local flow since 0 2 and 0 1 mm electrode diameter measured identical flow rates in the cortex of the same kidneys

The lower ratio OCF/ICF and higher ICF measured by 0 2 mm electrodes may to some extent be explained by the different relative depth of the deep cortical 0 2 and 0 5 mm electrodes The avera depths of the sampling zone in outer cortex for t two series were identical whereas the relati depth of the 0 2 mm electrode in inner cortex w smaller (12% of cortical thickness) than the 0.5 m electrodes For further information on normal d tribution of cortical blood flow we include unpu lished results from a previous study where t more evenly distributed sampling sites in differe parts of the cortex make it possible to divide cort into 4 zones (Fig 7) (Tyssebotn & Kirkebo 197 The figure indicates a continuous fall in blood fk from the surface of the kidney towards the co ticomedullary border and probably a more abru fall close to the border demonstrating that a ran of OCF/ICF ratios will be found as different ele trode depths are used

A gradual inward reduction of flow in corti agrees well with results published by Clausen et a (1978) using another diffusable indicator (^{125}I-an pyrine) and tissue sampling technique Howeve local cortical flow measurements with I-antipyrin were slightly higher than shown by H_2 clearance but the flow profile through the cortex was fairl similar The local cortical flow determined with th microsphere method varies from 3–7 ml/min g i outer cortex to 2–4 ml/min g in inner corte (McNay & Abe 1970 Clausen et al 1978) which in agreement with the present results using 0 2 m

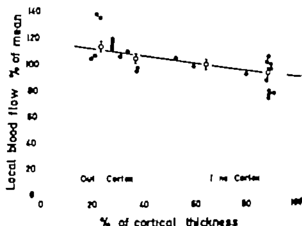


Fig 7 Blood flow at single electrode sites (± S E.) relate to the relative depth of the electrode i n in cortex in 1 expts (Tyssebotn & Kirkebo 1975) Open symbols giv average blood flow and electrode position in each quarte of cortex

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around the electrode tips An edema volume could be expected to increase progressively with time during vasodilator infusion but this pattern does not fit the steady increase of local flow demonstrated during the first 10 min of Ach infusion while RAF reached an almost stable level within 2 min

A further explanation for the varying response of single electrodes to dilation could be an uneven concentration of dilator agent due to incomplete mixing with renal arterial blood

Because H_2-saturated saline was injected simultaneously in the same catheter and large differences in electrode current might have been observed it is likely that only a part of the response variation can be explained in this way especially during Ach infusion However the complexity of the vasodilator/vasoconstrictor response to DA may well be related to different DA concentrations or uneven receptor distribution in different vessels causing a vasodilation in some areas and a partial constriction in others

Secondly changes in the kidney volume during vasodilation affect the clearance method and the electromagnetic method in different ways Local blood flow rate and RVF are calculated from H_2 desaturation curves directly in flow per unit actual volume ($F/V = \lambda \ln 0.5/T_1$) On the other hand RAF measured by arterial flow probe in ml/min is divided by the drained kidney weight determined at the end of the expt and no correction was made for changes in renal weight during vasodilation Omvik et al (1971) and Bang Olsen (1978) observed a 10–20% increase in renal volume when blood flow was raised 50–70% by Ach and bradykinin infusion Consequently when comparison of flow results is based on values for flow per unit volume the rise in RAF is probably overestimated by about 15% at maximal vasodilation partly explaining the greater rise in RAF than in local blood flow On the other hand although local flow showed a smaller rise than total flow absolute values for the RAF CFV and local blood flow measured by 0 2 mm electrode during maximal vasodilation were noticeably similar

Whether urine flow rises or not by Ach infusion (Aukland & Loyning 1970 Pinter et al 1964) the tubular fluid transport is small compared to cortical peritubular blood flow suggesting that the net tubular H_2 gas transport out of the cortex is negligible

In conclusion infusion of DA or Ach does not cause a redistribution of cortical peritubular blood flow Thus the increased natriuresis usually observed during vasodilation should not be explained by the effect of disproportionate increases in outer and inner cortical blood flow

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Relationships between peak force action potential duration and stimulus interval in rabbit myocardium

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WOHLFART B Relationships between peak force action potential duration and stimulus interval in rabbit myocardium. Acta Physiol Scand 1979 106: 395–409 Received 17 Nov 1978. ISSN 0001-6772. Department of Pharmacology University of Lund Sweden.

Isometric force and membrane action potential were recorded simultaneously in rabbit papillary muscles (36.5° 37.5°C) One to three test stimuli were given at various intervals (0 20–10 0 s) after a series of control contractions at constant stimulation intervals (1 0–1.5) Optimum peak force always occurred when the preceding test interval was 0.80 s. When this interval was >0 80 s, time to peak force was linear function of the action potential duration Furthermore, under these conditions the action potential duration (AP_1) and peak force (F_1) of the test contraction could be used to predict peak force (F_2) of the subsequent contraction elicited after a fixed interval (0.80–1.50 s) according to the equation (regression plane) $F = B_{AP} AP + B\ F + A$ Constants B_{AP} and B are interpreted to provide information about calcium influx during the action potential and of the recirculation of calcium between contractions, respectively F deviated towards higher values than predicted from the equation when the preceding test contraction was triggered to occur at an interval <0.80 This may be due to an intensified calcium transport into the cell during the action potential after these short intervals. The action potential duration was inversely related to both the inotropic state of the muscle (epresenting feed-back mechanism) and the preceding stimulation interval.

Key words Excitation-contraction coupling, myocardial contractility strength-interval relationships, postextrasystolic potentiation, calcium metabolism, sarcoplasmic reticulum

A fundamental feature of cardiac muscle is its ability to alter the contractile strength over a wide range. The present study has been aimed at elucidating the underlying mechanisms of this phenomenon by analysing the inotropic change that results from an altered stimulation interval (Bowditch 1871 Bravený & Kruta (1958) *Bravený Šumbera & Vaněk* (1969) postulated *that* the excitation interval determined the degree of *restitution* of the nearest following contraction and exerted a potentiating effect on the subsequent beats Blinks & Koch-Weser (1961) and Koch-Weser *&* Blinks (1963) interpreted the strength–interval relationship in terms of two opposing effects. According to them each contraction produces a positive and a negative inotropic effect with *different dissipation* rates.

It is now generally accepted that the amount of calcium being released into the myofibrillar space is the major determinant of the *contractile state of* the muscle (for a review see Fozzard 1977) *Edman & Johannsson* (1976) studying the force-frequency relationship in rabbit papillary muscle interpreted their results in terms of a two compartment model of the intracellular calcium metabolism. One of their compartments was concerned *with the release* of calcium into the *contractile system* while the other compartment resequestered the calcium It was assumed that a certain time must elapse between two stimuli in order to obtain a full release of calcium Their analysis showed that *the muscle s* capacity to produce force ("the optimum contractile *response*) *increases* steadily with the stimulation frequency *whereas* the steady-state force rises to a maximum and then declines.

Changes of the excitation interval also produce *variations* of the kinetics of the action potential (*Gibbs & Johnson* 1961 Edmands, Greenspan & Fisch 1966 Miller Wallace & Feezor 1971 Bass 1975 Anderson & Johnson 1976) These changes of the action potential ought to correlate with the mechanical events A linear relation between time to peak force and the action potential duration have been observed by many (see Bravený & Šumbera

ually shown by the inset of Fig. 4 1 a slightly modified protocol (inset of Fig. 2A) an extra stimulus was induced fixed time after the last control stimulus it was then followed by the two test stimuli in the same described. Thus the first test interval (in this case interval between the extra stimulus and the first test st) was varied in series while the second test interval was kept fixed

RESULTS

1 summarizes the significant contractile changes of the isolated papillary muscle that was seen when the pacing protocol (described under Methods) was used. It can be seen in the upper panel of Fig 1A that a short excitation interval (0.35 s) resulted in a contraction (designated 1) that was depressed and partly fused with the preceding contraction (designated "0") The following contraction (designated "2") was enhanced, a phenomenon known as postextrasystolic potentiation. A longer interval (0.50 s in the middle panel of Fig 1A) before contraction 1 gave a smaller depression of this contraction and also a smaller potentiation of contraction 2. When the interval was longer (5.0 s in the lower panel) than the interval between the regular stimuli (1.5 s) there was a increase of contraction 1 In this case contraction 2 was also reduced.

In Fig. 1B an extra contraction (designated "EC") has been imposed a fixed time after the last control contraction. It can be seen that this intervention increased the inotropic state of contraction 1. However by comparing the first panel of Fig. 1B with the second panel of Fig. 1A (in both of which the interval before contraction 1 is 0.5 s) it is evident that EC gave only a slight enhancement of contraction 2. The small potentiating effect of EC upon contraction 2 is also seen by comparing the two lower panels of Fig. 1A and 1B Here the contractions 2 have about the same magnitude Note also that this contraction is smaller than a control contraction. A further comparison between the middle and the lower panel of Fig. 1B reveals that the time for the decline of the contractile potentiation is shorter in the presence (middle panel) than in the absence (lower panel) of contractions (cf. Hoffman, Bindler & Suckling 1956).

Mechanical behaviour of the test contractions

Peak force of contraction 1 (Fig 2A) attained a maximum value when the first test interval was 0.80 s. This was the case both in the presence

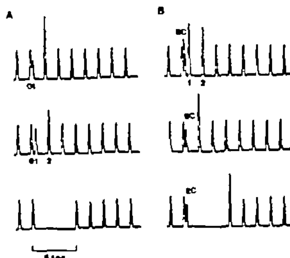


Fig 1 Paper-recordings of isometric contractions of an isolated papillary muscle illustrating the effects of altered stimulation intervals. For further information see text.

(upper two curves) and in the absence (lower curve) of a fixed extra contraction after the last control contraction Both shorter and longer intervals than 0.80 s caused a decline of peak force

The peak force of contraction 2 was strongly dependent on the setting of the first test interval (Fig 2B) The lower curve (no extra contraction) shows that there was an optimal interval for potentiation of contraction 2 This interval was 0.24±0 04 s (mean ± S D $n = 10$ preparations at basic stimulation intervals of 1.50 s) The maximum potentiation of contraction 2 ranged in the different experiments between 150 and 300% of control force (open triangles in Fig. 2) It is interesting to note that the introduction of the extra contraction caused only a slight potentiation of the peak force of contraction 2 (upper two curves of Fig. 2B)

The relation between the maximum speed of force development of contraction 1 (dF/dt) and the test interval is illustrated in Fig. 2C Maximum dF/dt of the enhanced contractions (upper two curves) was also reached at a test interval of 0 80 s However in the absence of an extra contraction (lower curve) dF/dt remained virtually constant for test intervals between 0.80 s and 10 0 s The concomitant decline of peak force as the interval was prolonged within this range (cf Fig. A) was attributable to a progressive decrease in duration of the contractions.

The maximum rate of force development of con-

1968 and Morad & Goldman 1973 for references) The action potential duration is also coupled to the force development of the subsequent contractions (Antoni Jacob & Kaufmann 1969 Braveny & Šum bera 1970 Edmands Greenspan & Bailey 1972) Moreover a feedback mechanism between the inotropic state and the action potential duration seems to operate within a contraction (Bass 1975 Bassingthwaighte Fry & McGuigan 1976) The relation between action potential and contraction thus appears to be complex The present study will show however that experimental conditions can be created where either the action potential duration or the peak force can be varied over a wide range while keeping the other variable constant Using this approach it was found that the excitation interval per se has a strong influence upon the coupling between the electrical and the mechanical events

METHODS

Preparation and mounting Papillary muscles from the right ventricle of (0.3–1 0 Kg) rabbits were used Immediately after killing and exsanguination the heart was removed and placed in prewarmed (about 35°C) oxygenated solution The right ventricle was split open and the dissection was carried out under a Zeiss stereo III microscope at 10 × magnification Thin (0 30–0 60 mm) and short (2–4 mm) papillary muscles were selected and excised A loop of platinum wire (diameter 0 25 mm) was tied (braided silk thread Ethicon 6/0) to the tendon of a selected muscle *in situ* The muscle was then cut free together with the piece of the ventricular wall to which it inserted and was pinned down to a cork sheet in the bottom of a dissection trough The ventricular block was trimmed to a size slightly wider than the base of the muscle and a U-shaped loop of platinum wire was tied to it. The muscle was mounted horizontally in a bath between two glass hooks one of which was connected to a force transducer The position of the other hook could be adjusted by means of a micrometer screw To minimize the mechanical artifacts on the action potential recording the preparation was sometimes supported from below by a glass rod.

Perfusion The bath was continuously perfused with oxygenated (95% O_2 and 5% CO_2) solution at a rate of about 4 ml per minute The temperature was thermostatically controlled (±0.2°C) and was between 36.5°C and 37.5°C in the different experiments The solution had the following composition (mM) NaCl 100 KCl 4 0 MgSO 1 5 CaCl 2.0 $NaHCO_3$ 20 NaH_2PO 1.5 Na-acetate 20 glucose 10 insulin 2 U/l pH was 7 45 All chemicals were of analytical grade and were dissolved in de-ionized water (double distilled in borosilicate glass)

Stimulation The muscle was stimulated to contract by passing current from a Grass S-44 stimulator through a pair of platinum wire electrodes placed near the base of the preparation The stimulus pulse was a unidirecti square wave (2 ms duration 150% of the threshold v for excitation) Under basic conditions the muscle paced at constant frequency At selected times a determined stimulus pattern (see below) was introdu For this purpose a specially constructed and progra able control unit was used to gate the Grass stimulato

Recordings of the mechanical and the electrica sponses The isometric force was recorded by mean strain gauge force transducer with a linear electrica sponse up to at least 20 mN Conventional glass mi electrodes having long and flexible shanks were u They were filled with 2.5 M KCl by boiling under redu pressure Later filling was performed from a coarser p pipette inserted into the capillary of the microelectr The electrometer had a high input impedance and provided with a capacitance neutralization unit. The f signal together with the membrane potential was cont ously displayed on a dual beam Tektronix 502 A osc scope The signals were also monitored on a polyg jet ink writer (Elema-Schönander Mingograf) at a paper speed The first derivative of the tension signal sometimes recorded on the paper Selected action po tial and the associated myograms were displayed o Tektronix 5103N storage oscilloscope and later ph graphed on 35 mm orthochromatic film with a Co camera.

Film analyses Each frame contained a control c traction (at steady-state) together with superimpo recordings of two test contractions (after varied stim intervals) The pictures (see Fig. 3) were analysed i Nikon model 6C profile projector at 10 × magnificati The action potential duration (AP) was taken as the t from the stimulus to the repolarization phase at a defi voltage, 60 mV below the peak of a superimposed con action potential This way of defining the voltage (c responding to about −40 mV) was used in order to red the influence of artificial potentials (i.e tip potenti on the electrical signal The peak developed force (and the time to peak force (TPF) were also measur the latter as the distance from the recorded stimu point to the estimated peak of the contraction The val obtained were usually recalculated to percent of the c responding values of the control contraction in order compensate for variations of the controls during the periment (variation coefficient were about 10%)

Experimental procedure and pacing protocol Af being mounted the muscle was paced for one hour bef the actual experiment started During this period t preparation was stretched to about 95% of the optim length for force production

The preparation was stimulated with constant stimu tion intervals (corresponding to a frequency of 0.67 1 0 Hz) during a control period These contractions designated 0" in the analysis The control period was selected times interrupted by two test stimuli. The fi test interval (i e the interval between the last cont stimulus and the first test stimulus) was varied in a giv series (from about 0.2 to 10 s) while the second test interv (i.e the interval between the test stimuli) was fixed (0 8 1.5 s) The resulting test contractions are referred to 1 and 2" respectively This pacing protocol is sch

...ously shown by the inset of Fig. 4. In a slightly modified protocol (inset of Fig. 2A) an extra stimulus was introduced a fixed time after the last control stimulus and was then followed by the two test stimuli in the same way as described. Thus the first test interval (in this case the interval between the extra stimulus and the first test stimulus) was varied in a series while the second test interval was kept fixed.

RESULTS

Fig. 1 summarizes the significant contractile changes of the isolated papillary muscle that was seen when the pacing protocol (described under Methods) was used. It can be seen in the upper panel of Fig. 1A that a short excitation interval (0.15 s) resulted in a contraction (designated "1") that is depressed and partly fused with the previous contraction (designated "0"). The following contraction (designated "2") was enhanced, a phenomenon known as postextrasystolic potentiation. A longer interval (0.50 s in the middle panel of Fig. 1A) before contraction 1 gave a smaller depression of this contraction and also a smaller potentiation of contraction 2. When the interval was longer (3.0 s in the lower panel) than the interval between the regular stimuli (1.5 s) there was a decrease of contraction 1. In this case contraction 2 was also reduced.

In Fig. 1B an extra contraction (designated "EC") has been imposed a fixed time after the last control contraction. It can be seen that this intervention increased the inotropic state of contraction 1. However, by comparing the first panel of Fig. 1B with the second panel of Fig. 1A (in both of which the interval before contraction 1 is 0.5 s) it is evident that EC gave only a slight enhancement of contraction 2. The small potentiating effect of EC upon contraction 2 is also seen by comparing the two lower panels of Fig. 1A and 1B. Here the contractions 2 have about the same magnitude. Note also that this contraction is smaller than a control contraction. A further comparison between the middle and the lower panel of Fig. 1B reveals that the time for the decline of the contractile potentiation is shorter in the presence (middle panel) than in the absence (lower panel) of contractions (cf. Hoffman, Bindler & Suckling 1956).

Mechanical behaviour of the test contractions

Peak force of contraction 1 (Fig. 2A) attained a maximum value when the first test interval was 0.80 s. This was the case both in the presence

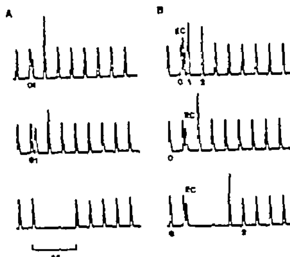


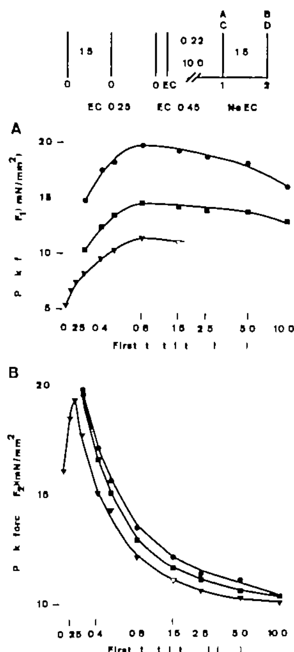
Fig. 1. Paper-recordings of isometric contractions of an isolated papillary muscle illustrating the effects of altered stimulation intervals. For further information see text.

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The peak force of contraction 2 was strongly dependent on the setting of the first test interval (Fig. 2B). The lower curve (no extra contraction) shows that there was an optimal interval for potentiation of contraction 2. This interval was 0.24 ± 0.04 s (mean ± S.D., n = 10 preparations at basic stimulation intervals of 1.50 s). The maximum potentiation of contraction 2 ranged in the different experiments between 150 and 300% of control force (open triangles in Fig. 2). It is interesting to note that the introduction of the extra contraction caused only a slight potentiation of the peak force of contraction 2 (upper two curves of Fig. 2B).

The relation between the maximum speed of force development of contraction 1 (dF/dt) and the test interval is illustrated in Fig. 2C. Maximum dF/dt of the enhanced contractions (upper two curves) was also reached at a test interval of 0.80 s. However, in the absence of an extra contraction (lower curve) dF/dt remained virtually constant for test intervals between 0.80 s and 10.0 s. The concomitant decline of peak force as the interval was prolonged within this range (cf. Fig. 2A) was attributable to a progressive decrease in duration of the contractions.

The maximum rate of force development of con-



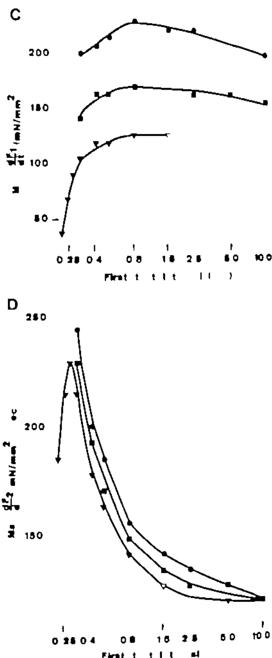


Fig 2A B Peak force (F and F_2) of the first (A) and of the second (B) test contraction related to the first test interval (on a logarithmic scale) The pacing protocol and the length of the intervals are shown in the inset The open triangles represent peak force of the control contraction

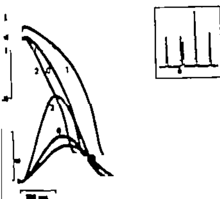
Fig 2C D The maximum rate of force develop (*dF/dt* and *dF₂/dt*) of the first (C) and of the secon test contraction in relation to the first test interval from the same experiment as shown in Fig. 2A B.

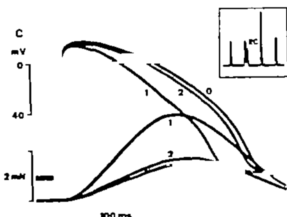
traction 2 (Fig 2D) was related to the first test interval in much the same way as was found for the peak force (cf Fig 2B) Thus there was a decline of *dF/dt* from a highly potentiated state to values below the control as the first test interval was increased from 0 26 s to 10 0 s The introduction of a fixed extra contraction caused only a minute enhancement of *dF/dt* (upper two curves)

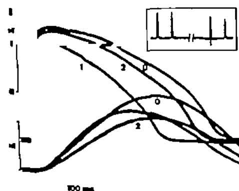
The contractile effects just described are fur analysed below in relation to the simultaneo recorded action potentials

Action potentials of the test contractions

The action potential of contraction 1 recorded a short test interval (0.23 s in Fig 3 A) exhibit greater overshoot and a longer duration tha







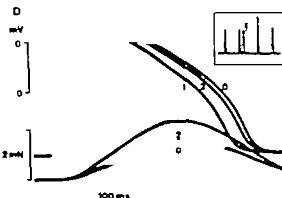


Fig 3 A, B Superimposed recordings of action potentials and isometric force from control contraction (0) and the two test contractions (1 and 2). The inset shows the same contractions in sequence. Basic stimulation intervals and second test interval 1.50 s. The first test interval A 0.23 and in B 5.0 s.

Fig 3 C, D Superimposed recordings of action potentials and isometric force from control contraction (0) and the two test contractions (1 and 2) which followed an extra contraction (EC). The sequence of the contractions is shown by the inset. Basic stimulation intervals and the two test intervals, 1.50 s. The interval before EC in C 0.21 and in D 0.40 s.

...tional registration. Opposite changes (reduced [illegible] and a shorter duration) were observed after a long first test interval (5.0 s in Fig. 3B). Potentiated contractions were often associated with a reduced action potential duration as exemplified by contraction 2 of Fig. 3A and also by contraction 1 of Fig. 3C and Fig. 3D. These features are analysed in further detail below.

The action potential durations were measured from pictures similar to those of Fig. 3 at a defined voltage (see Methods) close to −40 mV. This potential corresponds to the threshold value for activation of the slow inward current (Beeler & Reuter 1970, New & Trautwein 1972).

Fig 4A shows that the action potential of the first test contraction (AP) increased in duration to an optimum value (about 120% of the control) as the test interval was prolonged (0.18–0.25 s). A further lengthening (to 10.0 s) reduced AP (to 70% of the control). The results would thus seem to indicate that the action potential duration is strongly dependent on the length of the *preceding excitation interval*. Furthermore when the preceding interval was kept constant (this was the case with the second test contraction) an inverse relation between *contractile potentiation* and the action potential duration was seen. This inverse relation is evident by comparing the changes of AP (Fig. 4A) with the corresponding changes of *F* (Fig. 4B). Thus as the first test interval was prolonged from

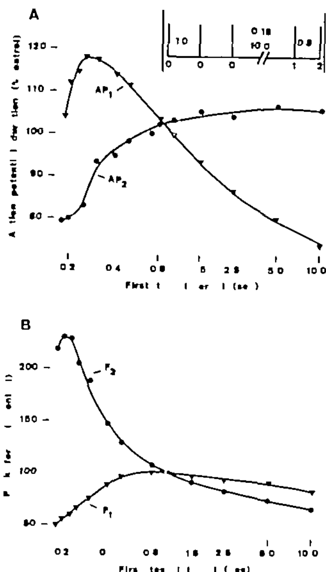


Fig 4 Action potential duration (AP and AP in A) and peak force (*F* and *F* in B) of the test contractions related to the first test interval (on a logarithmic scale) Variables are given in percent of the corresponding control valve (open triangles) Pacing protocol is shown by the inset.

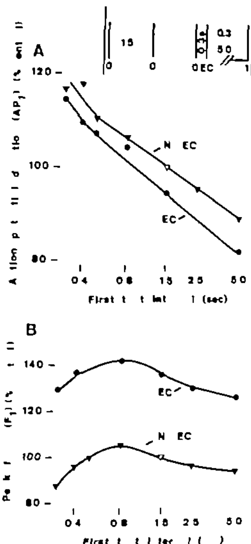


Fig 5 Action potential duration (A) and peak force (B of the first test contraction related to the first test interva in the presence (circles) and in the absence (triangles of a fixed extra contraction (EC) Pacing sequence is shown in the inset. Open triangles correspond to the control contraction.

0 20 s to 1.50 s AP_2 increased from 80% to 105% of the control At the same time F_2 declined from 230% to 90% of the control

The inverse relation between contractile potentiation and the action potential duration can also be inferred from Fig 5 Here AP (Fig. 5A) and *F* (Fig 5B) are plotted against the first test interval in the presence and in the absence of a fixed extra contraction (see inset) It can be seen that for any given value of the test interval the presence of the extra contraction increased *F* while AP was reduced

Time to peak force (TPF) related to action potential duration (AP)

The relation between TPF and AP was analysed in the two test contractions (pacing protocol of Fig 2A) A linear relationship between *TPF* and *AP* (Fig 6) was obtained for contractions elicited after ≥0 8 s intervals A relatively large scatter of the data points around the regression line (TPF on AP see Table 1) was generally seen The slope of the line was always smaller than 1 in the different experiments i e there was a smaller change of TPF than of AP The positive correlation between TPF

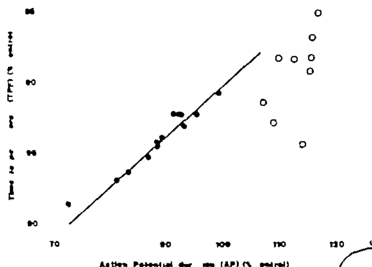


Fig. 6. Time to peak force (TPF) related to the action potential duration (AP) of the two test contractions. A regression line (TPF on AP) have been fitted to the data (expt. 403 in Table 1). Values shown by the open circles, referring to contraction 1 when the first test interval was shorter than 0.80 s, were not included in the regression analysis.

of AP was lost when the preceding interval was reduced below 0.8 s concomitant with the reduction of the contractile state of the muscle. Data from such contractions (open circles in Fig. 6) were therefore not included in the regression analysis.

Peak force of contraction 2 (F_2) related to action potential duration of contraction 1 (AP_1)

It is evident from the results presented in Fig. 4 that the peak force (F_2) of contraction 2 showed a similar relation to the first test interval as did the action potential duration (AP_1) of contraction 1. Both F_2 and AP_1 increased when the first test interval was shortened from 10.0 s and attained an optimum value for a short test interval. However the test interval for optimum AP_1 was longer than the interval for optimum F_2 (0.25 s and 0.20 s respectively). In the experiment demonstrated in Fig. 4)

Fig. 7 A, which is a replotting of the data illus-

Table 1. *The slopes (± S.D.) of the regression plane (F_2 on AP_1 and F_1) and of the regression line (TPF on AP) in the various experiments together with the correlation coefficients ( ) the number of measurements (n) and the basic stimulation frequency*

The equations are $F_2 = B_{AP} AP_1 + B_F F_1$ + const. and TPF = C_{AP} AP + const. All variables are expressed in percent of the corresponding control value

Expt.	B_{AP} ± S.D	B_F ± S.D			Stim. fr (Hz)	C_{AP} ± S.D		
[illegible]	0.58±0.05	0.28±0.02	0.99	11	1.00	0.48±0.07	0.85	23
[illegible]	1.20±0.09	0.12±0.01	0.98	10	1.00	0.33±0.03	0.93	24
[illegible]	1.34±0.10	0.28±0.02	0.97	15	1.00	0.52±0.04	0.93	31
[illegible]	0.33±0.04	0.35±0.03	0.97	15	1.00	0.30±0.03	0.86	39
[illegible]	0.86±0.03	0.24±0.01	0.99	13	1.00	0.40±0.02	0.96	35
[illegible]	1.41±0.19	0.16±0.03	0.93	12	0.67	0.48±0.04	0.87	39
[illegible]	0.56±0.04	0.38±0.03	0.99	14	0.67	0.34±0.03	0.88	37
[illegible]	1.03±0.08	0.26±0.02	0.98	15	0.67	0.53±0.03	0.93	41
Mean ± S.D	0.92±0.39	0.26±0.09				0.42±0.09		

[illegible]

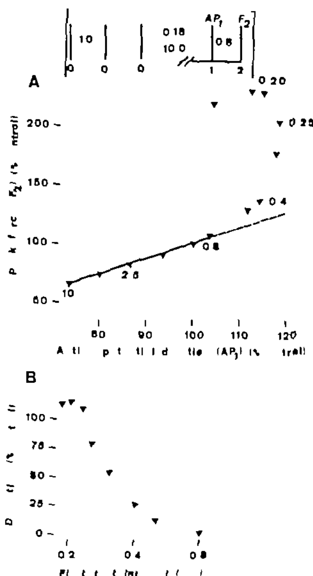


Fig 7 A Peak force (F) of contraction 2 related to *action potential duration (AP) of contraction 1* Figures refer to intervals before contraction 1 Replotted data from Fig 4 *B* The difference between the experimental value and the extrapolated (dotted in *A*) regression line (F on AP) plotted against the first test interval

trated in Fig. 4 shows the relationship between F_2 and AP_1 As can be seen F_2 was linearly related to AP_1 when data points referred to test intervals within the range 0 80–10 0 s (i e above the optimum test interval Fig 2 A) For values obtained after shorter test intervals than 0 80 s on the other hand the increase in peak force was greater than predicted (dotted line) from the linear part of the graph Fig 7B shows that the degree of deviation was increased with the shortening of the first test interval below 0 80 s It is of importance to note that the action potentials recorded after these short test intervals in addition to being of longer duration had an increased plateau voltage (cf contractio of Fig 3 A)

Peak force of contraction 2 (F_2) related to peak force of contraction 1 (F_1)

In studying the correlation between F_1 and F_2 duration of the action potential of contraction 1 kept constant This was done by varying both extra contraction interval and the first test inter relative to one another in an appropriate way Initial setting of the stimulus intervals was 0.20 s the extra contraction interval and 0 80 s for the f test interval The standard procedure was then increase the extra contraction interval stepw This would by itself increase AP_1 (by decreas the inotropic state of contraction 1 cf abov However this was compensated for by an app priate lengthening of the first test interval so as keep AP_1 constant The filled circles of Fig illustrates a series of measurements of this ki The filled squares of Fig 8 A represent anoth series of measurements in which the initial setti of the extra contraction interval and the first t interval was 0.20 s and 2 0 s respectively

Fig 8 B shows the relation between peak force contraction 2 and peak force of contraction 1 for t two fixed values of AP_1 As can be seen F_2 w linearly related to F in both the cases. For a given value of F the larger F_2 was obtained for t greater action potential 1

Fig 8 C D shows original recordings of t action potentials and the associated myograms the control and of the two test contractions from t experiment illustrated in Fig 8 A B Note that (the action potentials of contraction 1 had the sam duration and (2) that F_2 is larger when preceded b a larger F

Peak force of contraction 2 (F_2) related to both action potential duration (AP_1) and peak force (F of contraction 1

The relation between F_2 and AP (cf Fig 7 A) wa studied after the inotropic state of contraction 1 ha *been increased by the introduction of a fixed ext* contraction As can be seen in Fig 9 A this shifte the relation towards higher values of F_2 withou affecting the general shape of the relation In th plot illustrated in Fig 9 B the peak force of contrac tion 2 (F_2) have been replotted against both th action potential duration (AP) and the peak forc

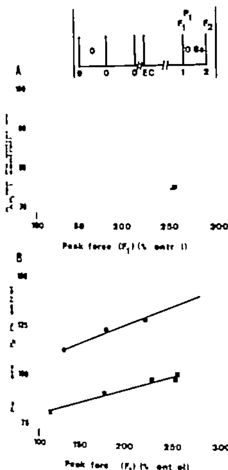


Fig. 8 A, B. *A*. Action potential duration (AP) plotted against peak force (F_1) of contraction 1. In the two experimental series the extra contraction interval and the first test interval were varied (see text) so as to keep AP constant. *B*. Peak force (F_2) of contraction 2 elicited after a fixed interval, see inset) related to peak force (F_1) of contraction 1. The regression lines (F_2 on F_1) are drawn.

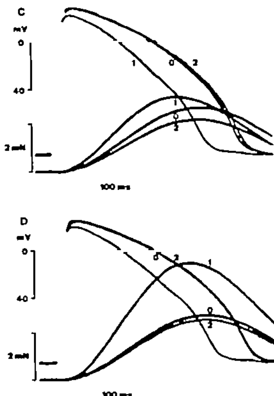


Fig. 8 C, D. Original records from the experiment of Fig. 8 A, B showing action potentials and isometric force from the two test contractions (1 and 2) superimposed on control registration (0). Extra contraction interval and first test interval in C. 0.50 and 3.0 and in D: 0.30 s and 2.30 respectively.

F_1 of contraction 1. The deviating values recorded after a shorter test interval than 0.80 s (right hand of Fig. 9 A) have been excluded in Fig. 9 B. It can be seen that the relation between F_2, AP and F_1 can be described by a regression *plane*. The equation for the plane was determined by using the least squares method for deviations around F_2. It is seen from the slope of the plane that increasing values of AP and F_1 both resulted in higher values of F_2.

The regression coefficients for the slope of the plane obtained in the various experiments are given in Table 1 together with the standard errors of the two slopes (F_2 relative to AP and to F_1). As can be seen the slopes varied considerably between the different experiments. However a comparatively small standard error was obtained within any given experiment. This is also reflected by the correlation coefficients which were close to 1.

DISCUSSION

The transient mechanical and electrical events that followed from a change of the excitation interval have been studied in the isolated papillary muscle

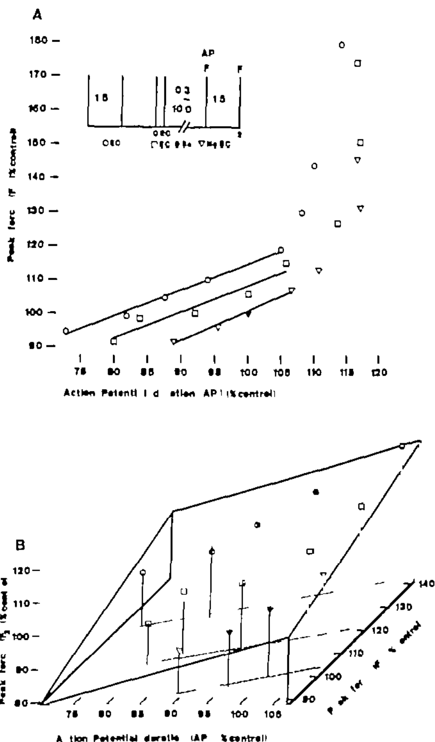


Fig 9 A Peak force (*F*) of contraction 2 in relation to action potential duration (AP) *of contraction 1* The different symbols *represent individual experimental series* as shown by the inset Filled triangle refers to the control value The regression lines (*F* on AP) for the linear part of the graphs are drawn (expt. 403 in Table 1) *B* Peak *force of* contraction 2 (F_2) related to both action potential duration (AP) and peak force (*F*) of contraction 1 A regression plane (*F* on AP and *F*) corresponding to the linear part of the graphs in A is illustrated The symbols represent the experimentally determined values whereas the vertical lines end at the calculated plane Dotted lines combine points within an experimental series

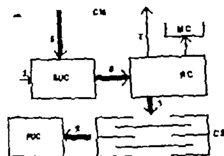


Fig. 11 Proposed model for the calcium metabolism in the E-C coupling (see text).

Five factors were found to be correlated with peak force of a given contraction. (1) The preceding excitation interval. (2) The duration of the concomitant action potential. (3) Action potential duration of the preceding contraction. (4) Tension development of the preceding contraction. (5) The preceding excitation interval.

According to current views calcium ions play a significant part in excitation-contraction coupling. The amount of calcium being released into the myofibrillar space is considered to be a major determinant of the inotropic state of the muscle. The five factors are discussed in the following in terms of a simple model of the metabolism of calcium in the excitation-contraction process. The model is a further development of the two-compartment system discussed by Edman & Jóhannsson (1976). Many related models have been proposed (Wood, Heppner & Weidmann 1969, Wussling & Szymanski 1972, Kaufmann et al. 1974, Allen, Jewell & Wood 1976, Langer, Frank & Brady 1976).

Proposed model of calcium metabolism in E-C coupling

Activator calcium is assumed to be stored in a compartment (RC of Fig. 10) from which it is released (arrow 1) in response to the action potential. The released calcium is taken up by the contractile element (CE) resulting in mechanical activation. Relaxation occurs as calcium is sequestered (arrow 2) by a primary uptake compartment (PUC). A major part of this calcium is transported (arrow 3) to a secondary uptake compartment (SUC) whereas the major part is assumed to be extruded (arrow 4) across the cell membrane (CM). This efflux is compensated for by the calcium entering the cell (arrow 5) during the action potential. This calcium goes into SUC. The transfer of calcium ions from SUC to RC (arrow 6) is assumed to be time dependent i.e. the refilling of RC between the releases requires a certain time to be completed. A small leakage (arrow 7) from RC out of the cell is also postulated in the model. This leakage will occur until an equilibrium between RC and the extracellular space is reached. A membrane compartment (MC) is supplied with calcium as a function of time. The filling rate of MC is assumed to be dependent on the cellular contents of contractile calcium. This dependence is symbolized by the dotted arrow from RC to MC. No attempt is made to identify the compartments of the model with any specific cellular structure. The model should merely be regarded as one possible mechanism by which calcium may serve as a regulator of the contractile activity.

The 5 factors (see above) and also 2 other factors correlated with the action potential duration will be discussed below together with the proposed model.

I *Factors related to peak force*

(1) *Relation to the preceding excitation interval.* The results have shown (Fig. 2A, Fig. C, Fig. 4B and Fig. 5B) that the optimum contractile response was recorded when the preceding excitation interval was 0.80 s (also see Edman & Jóhannsson 1976). This was the case in the presence as well as in the absence of a preceding fixed extra contraction. This optimum interval is explainable by the model if it is assumed that the amount of calcium in RC at the time of excitation determines the magnitude of the release. Between the releases RC is gradually refilled from SUC by a time dependent transport. However, there is also a small leakage of calcium from RC out of the cell. The interval for the optimum contractile response represents the time needed for peak concentration of calcium in RC to be attained. After this point the calcium contents of RC will gradually decrease.

(2) *Relation to action potential duration of the same contraction.* Changes of time to peak force (TPF) may affect the amplitude of a contraction. This is demonstrated by contractions 0 and 1 in Fig. 3B. TPF was linearly related to the action potential duration (AP) provided that the preceding excitation interval was >0.8 s. This correlation suggests that the time course of the calcium release is coupled to the action potential duration. However

the scattering of data points around the regression line (Fig 6 Table 1) indicates that AP is not the sole determinant of TPF Furthermore the relation between AP and TPF did not hold true for contractions recorded after shorter excitation intervals than 0 8 s (open symbols of Fig. 6) This may be attributed to the fact that the calcium replenishment of RC is not complete after these short excitation intervals leading to a reduced release

The decrease in action potential duration that occurs at test intervals >0 8 s (Figs 4 5) will reduce TPF and therefore reduce the peak isometric force This reduction of TPF will contribute (cf factor 1) to the decline in peak force after the attainment of optimum contractile response

(3) *Relation to action potential duration of the preceding contraction* Peak force (F_2) of contraction 2 increased linearly with the duration of the preceding action potential (AP) when the first test interval was shortened from 10 0 s to 0 80 s (Fig 7 A and Fig 9 A) However under these conditions peak force (F_1) of contraction 1 also varied When the variations of F were taken into account by relating F_2 to both AP_1 and F a regression plane was obtained (Fig. 9 B) This analysis shows that for any fixed value of F there exists a straight relationship between F_2 and AP_1 This relationship is understandable in terms of the model An increased action potential duration may be assumed to increase the calcium contents of *SUC* due to a longer duration of the calcium influx (arrows 5 Fig. 10) This calcium has to be transported to RC before it can be utilized in the excitation-contraction coupling. This means that the calcium entering with action potential 1 will be released from RC during contraction 2

The linear relationship between F and AP did not hold true when the first test interval was reduced below 0 80 s (Fig. 7) Under these conditions an increment of AP_1 caused a proportionally larger increment of F_2 One possible explanation for this phenomenon is that the calcium intake during these action potentials is intensified This accords with the findings reported by Hiraoka & Sano (1976) who measured the slow inward current under voltage clamp conditions in dog ventricular muscle They recorded a higher peak of the slow inward current in a premature contraction compared to the steady state contractions It is of interest to note in this connection that the action potential after these short intervals (cf Fig 3 A) had a higher amplitude in addition to a longer lasting oversh[illegible] than the control action potential A further possi[illegible] mechanism for the high value of F_2 when the f[illegible] test interval was shorter than 0 80 s will be [illegible]cussed under factor 5 below

The estimated calcium current during the act[illegible] potential as indicated by voltage clamp experime[illegible] is however considered too small to quantitativ[illegible] account for the force development This has be[illegible] discussed by Langer (1976) who proposed an el[illegible]troneutral calcium influx in addition to the calci[illegible] current which is measurable by the voltage cla[illegible] techniques The presence of such a mechanis[illegible] would fit into the present analysis

(4) *Relation to tension development of the pr[illegible]ceding contraction* The action potential duratio[illegible] (AP_1) of contraction 1 could be kept constant b[illegible] varying the two foregoing stimulus intervals (Fi[illegible] 8 A) Under these conditions peak force (F_2) of con[illegible]traction 2 was linearly related to peak force (F[illegible] of contraction 1 (Fig 8 B) This linear relationshi[illegible] is also evident from the regression plane (Fig. 9 B) for any fixed value of AP This finding can be interpreted to mean that a certain amount of th[illegible] calcium released in contraction 1 is being recircu[illegible]lated and released again in contraction 2 The magnitude of the recirculation (arrow 3 of Fig. 10) will be a positive function of the amount of calcium tha[illegible] was released from RC during the preceding contrac[illegible]tion The mean slope of the relation F_2 on F_1 (B_F i[illegible] Table 1) was 0.26±0 09 (± S D) This would mean in terms of the model that approximately one fourth of F is produced by calcium recirculated from the preceding contraction This rather small recirculation of Ca is noteworthy It would mean that a contraction is relatively more dependent on the externally derived calcium (arrow 5 of Fig 10) It would also mean that there is a great outflow of calcium with each contraction (arrow 4 Fig 10)

(5) *Relation to the pre-preceding excitation interval* It has been suggested above (factor 3) that action potentials elicited after shorter excitation intervals than 0 80 s are associated with an intensified calcium influx Another mechanism for the postextrasystolic potentiation is inherent in the model If a contraction is triggered to occur after a suboptimum interval the release is reduced because of a smaller amount of calcium in RC (cf factor 1) The calcium that is not released in the premature contraction i e the calcium still located in *SUC* will add to the intensified calcium influx during the

…tion potential and in this way contribute to the decrement of the following contraction (cf. Kaufmann et al. 1974).

The quantitative estimation of peak force of contraction 2

When peak force of contraction 2 (F_2) was analysed in relation to both peak force (F_1) and the action potential duration (AP_1) of contraction 1, a regression plane (Fig. 9B) was obtained:

$$F_2 = B_{AP} AP_1 + B_F F_1 + A$$

The slope of the plane is given by B_{AP} (seen from the AP-axes) and B_F (seen from the F_1-axes). The relation was valid when the first test interval was >1.0 s and the interval preceding contraction 2 was fixed. The equation can be regarded as the sum of two linear functions namely:

$$F_2 = B_{AP} AP_1 + A$$
$$F_2 = B_F F_1 + A$$

where B_{AP} denotes the effect of a change of AP upon F_2 (arrow 5 of Fig. 10) and B_F denotes a change of F_1 upon F_2 (arrow 3 of Fig. 10). Assuming that A is zero B_F is a measure of the recirculation of calcium between contractions. The parameters (B_{AP} and B_F) could be estimated by varying one of the variables (AP_1 and F_1) while the other variable was kept constant (cf. Fig. 8). However provided that AP_1 and F_1 are not linearly dependent in a set of experimental data, multiple linear regression analysis can be used for the estimation of the parameters. This approach had the advantage that the analysis did not require that one of the variables was kept constant.

An examination of the mean value of the parameters (given in Table 1) shows that a 10% change of AP_1 (F_1 fixed) was accompanied by an almost equal percentage change of F_2. A change of F_1 on the other hand resulted in a four times smaller change of F_2. The standard deviation within a given experiment was considerably smaller than that obtained between the experiments. This means that the relation between F_2, AP_1 and F_1 could be well described by a plane. However the slope of the plane was different in the different preparations.

II. Factors related to action potential duration

The model presented in Fig. 10 contains a membrane compartment (MC) to account for the observed changes in action potential duration. It is postulated that a calcium release takes place from MC during the excitation-contraction cycle. The replenishment of MC is assumed to occur between the excitations by a time dependent calcium transport, the magnitude of which is dependent on the cellular contents of contractile calcium. It is furthermore postulated that the action potential duration is inversely related to the calcium contents of MC (cf. Bravený & Šumbera 1970, Bass 1975, Bassingthwaighte, Fry & McGuigan 1976).

(1) *Relation to the preceding excitation interval.* From the postulated time dependence of the filling of MC it follows that the calcium contents of MC at the time of excitation become smaller as the excitation interval is decreased. Thus when the first test interval is reduced below 10.0 s, AP will increase (cf. Fig. 4A and Fig. 5A). The decrease of the action potential duration that occurred after very short stimulus intervals (0.20–0.30 s) may be attributed to an incomplete recovery of the ion currents just after an action potential.

(2) *Relation to the inotropic state.* The inverse relationship between contractile potentiation and the action potential duration that was observed in contraction 1 (Fig. 5) and contraction 2 (Fig. 4) is also explainable by the model. An increased calcium loading of RC, as reflected by the contractile potentiation, will lead to a more intense calcium transport to MC. The increased calcium contents of MC will lead to reduction of the action potential duration.

This inverse relationship constitutes a stabilizing feed-back mechanism. If for instance in a sequence of contractions under steady-state conditions the inotropic state of one of the contractions would increase (decrease), this would lead to a shortening (lengthening) of the concomitant action potential. The resulting reduction (increment) of the calcium intake would tend to bring back the inotropic state of the following contraction towards the original value.

III. Interpretation of contractile behaviour of heart muscle based on proposed model

This section summarizes events which may underlie the contractile changes (see Fig. 1) that are associated with an alteration of the stimulus interval. Reference is made to the preceding sections where the various factors have been discussed.

The contractile potentiation seen after a short

the scattering of data points around the regression line (Fig 6 Table 1) indicates that AP is not the sole determinant of TPF Furthermore the relation between AP and TPF did not hold true for contractions recorded after shorter excitation intervals than 0 8 s (open symbols of Fig 6) This may be attributed to *the fact that the calcium replenishment* of RC is not complete after these short excitation *intervals leading to a reduced release*

The decrease in action potential duration that occurs at test intervals >0 8 s (Figs 4 5) will reduce TPF and therefore reduce the peak isometric force This reduction of TPF will contribute (cf factor 1) to the decline in peak force after the attainment of optimum contractile response

(3) *Relation to action potential duration of the preceding contraction* Peak force (F_2) of contraction 2 increased linearly with the duration of the preceding action potential (AP_1) when the first test interval was shortened from 10 0 s to 0 80 s (Fig. 7 A and Fig 9 A) However under these conditions peak force (F_1) of contraction 1 also varied When the variations of F_1 were taken into account by relating F_2 to both AP and F a regression plane was obtained (Fig 9 B) *This analysis shows that for* any fixed value of F_1 there exists a straight relationship between F_2 and AP_1 *This relationship is under*standable in terms of the model An increased action potential duration may be assumed to increase the calcium contents of SUC due to a longer duration of the calcium influx (arrows 5 Fig 10) This calcium has to be transported to RC before it can be utilized in the excitation-contraction coupling. This means that the calcium entering with action potential 1 will be released from RC during *contraction* 2

The linear relationship between F and AP did not hold true when the first test *interval was re*duced below 0 80 s (Fig 7) Under these conditions an increment of AP caused a *proportionally larger* increment of F_2 One possible explanation for this phenomenon is that the cal ium intake during these action potentials is intensified This accords with the findings reported by Hiraoka & Sano (1976) who measured the slow inward current under voltage clamp conditions in dog ventricular muscle They recorded a *higher* peak of the slow inward current in a premature contraction compared to the steady state contractions It is of interest to note in this connection that the action potential after these short intervals (cf Fig. 3 A) had a higher amplitude in addition to a longer lasting overshoot than the control action potential A further possible mechanism for the high value of F_2 *when the first test interval* was shorter than 0 80 s will be discussed under *factor 5 below*

The estimated calcium current during the action *potential as indicated by voltage clamp experiments* is however considered too small to quantitatively account for the force development This has been discussed by Langer (1976) who proposed an electroneutral calcium influx in addition to the calcium current which is measurable by the voltage clamp techniques The presence of such a mechanism would fit into the *present analysis*

(4) *Relation to tension development of the preceding contraction* The action potential duration (AP) of contraction 1 could be kept constant by varying the two foregoing stimulus intervals (Fig. 8 A) Under these conditions peak force (F_2) of contraction 2 was linearly related to peak force (F_1) of contraction 1 (Fig 8 B) This linear relationship is also evident from the regression plane (Fig. 9 B) for any fixed value of AP_1 This finding can be interpreted to mean that a certain amount of the *calcium released in contraction 1 is being recircu*lated and released again in contraction 2 *The magnitude of the recirculation (arrow 3 of Fig 10) will* be a positive function of the amount of calcium that was released from RC during the preceding contraction The mean slope of the relation F_2 on F_1 (B_F in Table 1) was 0 76±0 09 (± S D) This would mean in terms of the model that approximately one fourth of F_2 is produced by calcium recirculated from the preceding contraction This rather small recirculation of Ca is noteworthy It would mean that a contraction is relatively more dependent on the externally derived calcium (arrow 5 of Fig 10) It *would also mean that there is a great outflow of* calcium with each contraction (arrow 4 Fig 10)

(5) *Relation to the pre-preceding excitation interval* It has been suggested above (factor 3) that action potentials elicited after shorter excitation intervals than 0 80 s are associated with an intensified calcium influx Another mechanism for the postextrasystolic potentiation is inherent in the model If a contraction is triggered to occur after a suboptimum interval the release is reduced because of a smaller amount of calcium in RC (cf factor 1) The calcium that is not released in the premature contraction i e the calcium still located in SUC will add to the intensified calcium influx during the

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pre preceding excitation interval (postextrasystolic potentiation) The premature contraction (i e the contraction elicited after a short excitation interval) may involve the following events.

(*a*) An intensified calcium influx (section I 3)

(*b*) An increased *duration* of the calcium influx as indicated by the prolonged action potential (section I 3)

Both *a* and *b* will lead to greater amount of calcium in RC at the moment of the *subsequent* stimulus

(*c*) Some calcium is stored in SUC when the premature stimulus is applied This calcium will have reached RC to be released by the following stimulus (section I 5)

The contractile depression seen after a long pre-preceding excitation interval The contraction triggered after a long excitation interval may involve the following events

(*a*) A reduced calcium intake as indicated by the shorter action potential duration (section I 3)

(*b*) A smaller amount of calcium in RC due to a longer lasting outflow during the preceding excitation interval (section I 1)

As a result of *a* and *b* there will be a reduced quantity of calcium in RC when the next stimulus occurs

The decay of the postextrasystolic potentiation Activation of the muscle caused a rapid decay of the potentiated contractile state (Fig 1 contractions 2 of Fig 3C D) This may involve the following events

(*a*) The reduced action potential duration of the enhanced contraction will lead to a reduced calcium influx into the cell to be used in the following contraction (section I 3)

(*b*) The recirculation of calcium after a contraction is assumed to be small This would mean that a contraction is relatively little potentiated by a previous enhanced contraction (section I 4)

(*c*) Each activation of the muscle leads to a great outflow of calcium Between contractions the outflow of calcium is assumed to occur very slowly This can explain why the time course for the decline of the potentiation was faster in the presence than in the absence of contractions (cf middle and lower panels of Fig 1B)

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Myogenic microvascular responses to change of transmural pressure

A mathematical approach

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The recently described static and dynamic myogenic responses in the sympathectomized skeletal muscle microvessels to given transmural pressure (P_T) change applied at different rates (dP_T/dt) (Grände & Mellander 1978), were further analysed in this study with mathematical approach. The hypothesis that myogenic reactions are triggered by and related to wall tension was also tested. The mathematical model was based on a force-equilibrium in the microvessel wall including passive forces related to vascular transmural pressure, elasticity and wall-viscosity and active myogenic forces related to wall tension and its rate of change. Great resemblance was demonstrated between microvascular resistance curves obtained with the model and corresponding curves observed in vivo indicating that the model quite adequately can describe myogenic microvascular resistance responses to transmural pressure stimuli. The results support the myogenic hypothesis in general and, in particular the concept of an important rate sensitivity in myogenic microvascular control and are compatible with the view that myogenic reactions are triggered by and related to change of wall tension. The model in addition, provided data for certain microvascular variables which are difficult to assess by in vivo observations, e.g. Young modulus of elasticity wall tension, its rate of change, and internal vessel radius, and it offered means to define more precisely the role of physical factors like effects of Poiseuille and Laplace laws in vascular resistance regulation.

Key words: Myogenic vascular control, mathematical model, mechanics of microvessels, elasticity computer-simulation, rate-sensitivity transmural pressure change autoregulation

The hypothesis of Bayliss (1902) of enhanced contractile activity in vascular smooth muscle in response to increased blood pressure has received strong experimental support from hemodynamic whole organ and vital microscopy studies (e.g. Folkow 1949 1964 Wiedeman 1963 Mellander et al. 1964 Baez et al. 1974 Johnson & Intaglietta 1976). The myogenic responses described in those investigations were almost exclusively studied in terms of the active constrictor/dilator responses which develop upon a given steady-state increase/decrease in vascular transmural pressure. More recent studies have demonstrated that myogenic activity is much more vivid during the phase of changing transmural pressure indicating a rate-sensitive dynamic' component in myogenic vascular control.

The existence of rate-sensitivity in myogenic regulation was evidenced by in vitro observations of electrical and mechanical activity in isolated single-unit vascular smooth muscle exposed to a given passive stretch or shortening applied at various rates of length change (Johansson & Mellander 1975). Static stretch was shown to evoke only a moderate excitatory response above the resting control value while dynamic stretch was much more effective causing marked excitatory responses graded in relation to the rate of length change. Pas-

With seemingly reasonable numerical values for the different quantities in the equation the microvascular resistance curves derived from the mathematical model showed great resemblance to corresponding resistance curves observed in skeletal muscle in vivo in response to given changes of P_T and dP_T/dt. This similarity between results obtained with two entirely different approaches can be taken as additional support for the concept of an important wall tension dependent rate-sensitivity in myogenic microvascular control. The mathematical approach also offered numerical data for previously poorly defined variables, such as Young's modulus of elasticity, changes of wall tension, changes of internal vessel radius etc.

MATHEMATICAL MODEL

Mechanics of an average microvessel

The average myogenically reactive microvessel is considered to be a cylindrical tube specified in the following way (Fig. 1):

L, tube length
$r(t)$, internal tube radius, where t is time
r_0, internal tube radius representing relaxed vessel at zero transmural pressure (experimentally tested at close to zero arterial and at zero venous pressure, see below)
internal tube radius representing vessel at normal distending arterial and venous pressures and normal vascular tone
$h(t)$, wall thickness
α, fraction of $h(\ )$ containing active tension
h_0, wall thickness at radius
h_1, wall thickness at radius
ρ, wall density
$\sigma(t)$, wall tension
$P(t)$, transmural pressure

For simplicity an arbitrary function $f(t)$ and its first, second, and third time derivatives will be denoted f, $\dot{f}$, $\ddot{f}$ and $\dddot{f}$.

Since wall volume (wall volume $= L\cdot\pi\cdot[2r+h(\ )]h(\ )$) can be considered constant during changes of tube radius, wall thickness $h(r)$ can be related to internal radius as follows:

$$h(r) \quad \sqrt{\quad} \quad h_1(h_1 \quad 2r_1) \tag{1}$$

$h(r)$ will be denoted h below.

Force equilibrium in the radial direction for finite element of the cylindrical tube with the element angle $d\theta$ gives (Fig. 1B).

$$\rho h\ddot{r}\Delta L\theta \quad pr d\theta \Delta L \quad 2\sigma\alpha h \Delta L \quad \sin\frac{d\theta}{2} \tag{2}$$

where σ is defined as mean value across the contractile part of vessel wall of actual wall tension.

For small angles $\sin d\theta = d\theta$ hence

$$\sigma \quad \frac{pr}{\alpha h} - \frac{\rho r\ddot{r}}{\alpha} \tag{3}$$

The second term in eq. (3) can be neglected, since it is exceedingly small compared to the first one, wherefore eq. (3) describes the law of Laplace.

The following forces are assumed to operate in the tangential tube direction: F_E passive elastic force N F passive viscous force which according to Hook's law can be defined as

$$F \quad Lh \frac{\quad}{\quad + \frac{h_0}{2}} E, \tag{4}$$

where E is Young's modulus of elasticity assumed to be constant.

If

$$C \quad \frac{LE}{\quad + \frac{h_0}{2}} \tag{5}$$

where C is a constant

$$F = C_E h(\ -r_0) \tag{6}$$

F wall viscosity force assumed related to rate of wall movement

$$F \quad C\dot{r} \tag{7}$$

where C is a constant.

According to the myogenic hypothesis for microvessels proposed by Grände et al. (1977, 1978) altered transmural pressure evokes two active myogenic force components, in this model assumed to be related to wall tension, and its rate of change, viz.

F static myogenic force

$$F \quad C \tag{8}$$

and

F_D dynamic myogenic force

$$F_D \quad C\dot{\sigma} \tag{9}$$

where C and C are constants.

F contractile basal force at normal distending arterial pressure which is a constant active force representing the normal contractile state in the microvessel at control arterial pressure,

$$F \quad C \tag{10}$$

where C is constant.

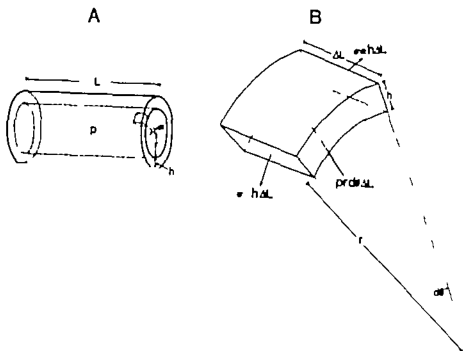


Fig 1 Panel A illustrates a thick walled tube representing an average microvessel with internal radius r wall thickness h length L exposed to a transmural pressure p Panel B demonstrates a segment element of this tube wall defined by the infinitesimal angle $d\theta$ and length ΔL Forces operating on this element can be expressed by its tangential force $\sigma\alpha h\Delta L$ (where σ is wall tension and α fraction of h consisting of contractile elements) and by its radial force $p\Delta Lrd\theta$ caused by transmural distending pressure p

sive shortening at different rates caused graded inhibitory responses below the control level to complete inhibition at the highest rates of shortening Further analysis suggested that the true dynamic myogenic stimulus was related to the rate of change of tension rather than rate of change of length (Sigurdsson et al 1977)

Rate-sensitivity in myogenic control has been evidenced also in the microvessels (<20 μm i d) in vivo (Grände Lundvall & Mellander 1977 Grände & Mellander 1978) from studies on the vascular bed of skeletal muscle exposed to a given change of vascular transmural pressure (P_T) applied at different rates (dP_T/dt) over a range from +7 5 to −7 5 mmHg/s The dynamic microvascular responses developing during the phase of changing P_T were found to be distinctly graded in relation to the magnitude of the dP_T/dt stimulus The static microvascular responses revealed in the steady state phase of constant increased P_T were small compared to the dynamic ones and graded in relation to the amplitude of the P_T increase Rate-sensitivity in microvascular control was bi-directional causing constriction in response to positive and dilation in response to negative values of dP_T/dt The more proximal arterial vessels (>20 μm i d) responded to static but not to dynamic myogenic stimuli whereas the large venous vessels seemed to lack myogenic reactivity entirely

The main purpose of the present study was to further test the hypothesis of rate sensitivity in myogenic microvascular control by using a mathematical approach which also permitted test of the hypothesis that the myogenic responses are triggered by change in wall tension Our mathematical model for microvascular reactivity is based on a force equilibrium in the microvessel wall including a force due to vascular transmural pressure a force related to elasticity a wall viscosity force related to the rate of wall movement and the active myogenically induced forces related to the above-mentioned static and dynamic responses to transmural pressure changes Data for morphological and physical quantities in the model were obtained from the current biological literature Solutions of this mathematical model in terms of microvascular resistance curves were obtained with the aid of a computer

[illegible] in vivo data from sympathectomized cat skeletal muscle (Grände & Mellander 1978) for Q AP SAP and SVP numerical values for k_e, C_e, C_T, and C can be obtained. In the control situation with basal vascular tone, AP averaged 105 mmHg, SAP 55 mmHg, SVP 15 mmHg, and Q 6.5 ml min 100 g giving proximal arterial resistance, R_{prox} of 7.7 PRU (mmHg ml min 100 g) and microvascular resistance R_{micro} of 6.2 PRU. Inserting in eq. (17) these in vivo data for the control situation, here gives

$$\frac{C_{micro}}{C_{prox}} = 2.7 \cdot 10$$

A numerical value for C_{micro} can be derived from eq. [illegible] using data for the control situation:

$$C_{micro} = 1.5 \cdot 10^{[illegible]} \text{ PRU } [illegible]$$

A value for can be roughly deduced from in vivo observations of R_{micro} in the paralysed vascular bed at close to zero transmural pressure (AP<10 mmHg, VP=0 mmHg). R_{micro} in this situation, denoted R_0, was found to be about 8.5 PRU (unpublished observations) wherefore according to eq. (16)

$$r_0 = [illegible] \cdot 10^{-4} m,$$

thus indicating according to eq. (4) the passive elastic force F_E to be directed outwards. It therefore will tend to increase vessel radius within the normal physiological range ($r<r_0$), (see Discussion). *Inserting this value in eq. (I) gives*

$$r_0 = 2.5 \cdot 10^{-4} m$$

A numerical value for Young modulus of elasticity (E) for the microvessel in its *normal physiological range* of radius variation is not available in the current biological literature, but the present model can offer means to estimate its magnitude and also to calculate the other model constants, viz. C, C_e, C and C by using an iterative method conventional in numerical analysis.

The starting value for E in this iterative process was chosen within the range given by Gore (1972). This value and the defined values for L and k_e were inserted in eq. (3) which gives starting value for C_e.

Starting values for C and C can now be calculated from eq. (14) for any steady-state situation in the vascular bed, where $p = r = 0$ which gives

$$\frac{C_1 ak \quad \dfrac{C_1 ak}{[illegible]}}{C\, ak \quad p(C_1 \; akL)} \tag{18}$$

according to the law of Poiseuille.

$$\frac{R_{micro}}{R_0} = \left[\frac{C_1 ak + p(C_1 - akL)}{C_1 ak - \dfrac{C_1 ak}{[illegible]}}\right] \tag{19}$$

where p in turn can be defined:

$$p = \frac{SAP + SVP}{2} + P_T, \tag{20}$$

where P_T is the experimentally produced change of overall vascular transmural pressure evoked by alteration of extravascular (plethysmographic) pressure (see Grände et al. 1977).

Starting values for C and C can now be derived from eq. (19) by inserting the following two known values for R_{micro} obtained in vivo (Grände & Mellander 1978) at two different mean microvascular distending pressures (p):

p=35 mmHg *for the control situation where* SAP=55 mmHg, SVP=15 mmHg, P_T=0 mmHg, and R_{micro}= 6.2 PRU

and

p=65 mmHg for a steady-state situation after an increase of P_T by 30 mmHg, where R_{micro} was increased by the *static myogenic response to 7.4 PRU. SAP and SVP* can be considered unchanged in this situation (Grände et al. 1977).

Calculation of the starting values for C and C from eq. (14) requires an expression for rate of change of transmural pressure ($\dot{p}$) which was obtained in the following way:

By assuming uniform volume flow of blood through the different sections of the arterial vessels and constant SVP (see above) the following expression for SAP was obtained (cf. Pappenheimer & Soto-Rivera 1948).

$$SAP = \frac{AP \dfrac{R_{micro}}{R_{prox}} + SVP}{1 + \dfrac{R_{micro}}{R_{prox}}} \tag{21}$$

This can be rearranged by using the expression for R_{micro}/R_{prox} in eq. (17) and by denoting $C_{micro}/C_{prox}=C_r$.

$$SAP = \frac{AP \; C_r\left(\frac{r_{prox}}{r}\right) \quad SVP}{1 \quad C_r\left(\frac{r_{prox}}{r}\right)} \tag{22}$$

Thus

$$\frac{SAP + SVP}{2} = \frac{(AP + SVP) \; C_r\left(\frac{r_{prox}}{r}\right) + 2SVP}{2\left[1 + C_r\left(\frac{r_{prox}}{r}\right)^4\right]} \tag{23}$$

A tangential force equilibrium

$$\sigma\alpha hL = F_E + F_V + F_s + F_D + F_C, \quad (11)$$

which after substitution from eq (6)–(10) gives

$$\sigma\alpha hL = C_e h\,(r-r_0) + C\,\dot{r} + C_s\sigma + C\,\dot{\sigma} + C \quad (12)$$

Differentiation of eq (3) gives

$$\dot{\sigma} = \frac{\dot{p}r}{\alpha h} + \frac{p\dot{r}}{\alpha h}\left(1 - \frac{r}{h}\,\frac{dh}{dr}\right) \quad (13)$$

where $dh/dr<0$ for all r (see eq 1)

Substituting eq (3) and (13) in eq (12) gives

$$\dot{r}\left[C + \frac{C\,p}{\alpha h}\left(1 - \frac{r}{h}\,\frac{dh}{dr}\right)\right] + r\left(C\,h + \frac{C_s p}{\alpha h} + \frac{C_s \dot{p}}{\alpha h} - pL\right) = C\,hr - C_t \quad (14)$$

Eq (14) thus describes mathematically internal radius (r) changes of the average myogenically reactive microvessel as specified above in response to change of transmural pressure (p)

Application of model to biology

Myogenic reactivity to transmural pressure changes has been studied in detail in the vascular bed of skeletal muscle in vivo (Grände et al 1977 1978) by simultaneous measurements of total regional resistance and segmental resistances along the vascular bed with a technique described by Grände & Borgström (1978) From continuous measurements of arterial blood flow (Q) mean arterial inflow pressure (AP) small artery pressure (SAP) small vein pressure (SVP) and venous outflow pressure (VP) (cf Haddy et al 1954) total resistance was represented by (AP−VP)/Q proximal arterial resistance by (AP−SAP)/Q microvascular resistance by (SAP−SVP)/Q and large vein resistance by (SVP−VP)/Q It was concluded from the two first mentioned studies that dynamic myogenic reactivity to a transmural pressure change was almost exclusively confined to arterial microvessels smaller than about 20 μm i d (cf Fronek & Zweifach 1975) These vessels also showed a static myogenic response to a steady state transmural pressure change and such static reactivity was present in the more proximal arterial vessels (>20 μm i d) as well The latter active response in the proximal arterial vessels roughly balanced out the passive distension effect of increased pressure wherefore these vessels largely behaved as rigid tubes to a transmural pressure change within given limits. The large venous vessels seemed to lack myogenic reactivity entirely

The present mathematical model of the vascular bed deals with the myogenically highly reactive microvessels discussed above The more proximal series-coupled arterial vessels will via overall flow alterations have significant influence on SAP and hence on mean distending microvascular pressure when transmural pressure is changed. Therefore the proximal vessels must be taken into consideration in our model Corresponding effects of flow alterations on SVP are much smaller (Grände et al 1977) and hence will have little influence on microvascular distending pressure and therefore SVP will be considered constant in the present model This implies that, for the present purpose the venous section can be neglected Similarly the true capillaries, which can be considered as rigid tubes with comparatively small resistance (Landis 1930) will be neglected in the model

As arterial vessels larger than 250 μm have negligible resistance (Fronek & Zweifach 1975) the vascular bed to be considered in the present model will be represented by proximal arterial vessels with internal radius varying from 250 μm down to 10 μm coupled in series with arterial microvessels with radius varying from 10 μm down to 5 μm In the mathematical model the proximal arterial vessels will be represented by a number of parallel-coupled cylindrical tubes assumed to be rigid during change of transmural pressure Their length is considered to be 20 mm (Wiedeman 1963*b*) and their radius (r_{prox}) 29 μm dimensions which make the cylindrical tube equi-resistant to a cone-shaped proximal arterial vessel as defined above The microvessels in turn are represented by a number of parallel-coupled reactive cylindrical tubes obeying differential equation (14) in response to a transmural pressure change The following data derived from the current biological literature may then apply to such tube

- L tube length 1 mm (in vivo range approximately 0.5–1.5 mm Wiedeman 1963*b* Eriksson & Myrhage 1972 Lipowski & Zweifach 1977)
- r internal tube radius at normal distending pressure μm (equi-resistant to a cone-shaped microvessel with internal radius decreasing from 10 to 5 μm along its length of 1 mm
- h wall thickness at radius 4 μm (Intaglietta & Tompkins 1972, Fronek & Zweifach 1975)
- α fraction of h sustaining active tension 0.5 (Caro et al 1978)
- ρ wall density 10^3 kg/m

Model tube resistance R can be calculated according to Poiseuille's law $R=(8\,\eta L/\pi n)\,(1/r)$ where η is blood viscosity L tube length n number of parallel-coupled tubes and r inner tube radius The term $8\,\eta L/\pi n$ was assumed to be a constant which for the proximal tubes was denoted C_{prox} and for the microtubes C_{micro} Hence

$$R_{prox} = C_{prox}\,\frac{1}{r_{prox}} \quad (15)$$

and

$$R_{micro} = C_{micro}\,\frac{1}{r^4} \quad (16)$$

giving

$$\frac{R_{micro}}{R_{prox}} = \frac{C_{micro}}{C_{prox}}\,\frac{r_{prox}}{r^4} \quad (17)$$

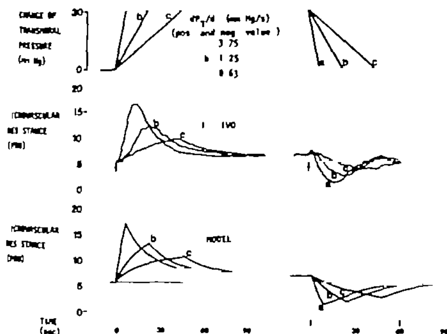


Fig. 2 Comparison between microvascular myogenic resistance responses obtained with the mathematical model and corresponding in vivo curves observed in the vascular bed of sympathectomized cat skeletal muscle. Note the dynamic constrictor responses evoked during phasic increase in vascular transmural pressure P_T of 30 mmHg applied at three different dP_T/dt rates, 3.75, 1.25 and 0.63 mmHg/s, the static responses evoked in the steady state of constant increased P_T and the dynamic dilator responses evoked by P_T decrease at the specified rates. The resistance tracings are reproduced on top of each other

RESULTS

The above described model for myogenic microvascular resistance responses to change of the transmural pressure was constructed for one arbitrary set of P_T and dP_T/dt values (30 mmHg 1.25 mmHg/s). In a first series of experiments the validity of the presented model was tested by comparing the model resistance curves with corresponding curves observed in vivo over a wide range of P_T values (from 0 to 40 mmHg) and dP_T/dt values (from −7.5 to +7.5 mmHg/s). The in vivo data were taken from the material previously presented by Grande & Mellander (1978).

Fig. 2 shows an example of such a comparison. The middle panel shows for one representative experiment how microvascular resistance in skeletal muscle in vivo was influenced by a given total increase in P_T of 30 mmHg applied at 3 different rates (dP_T/dt 0.63, 1.25 and 3.75 mmHg/s) and by a subsequent decrease in P_T back to control level at the same given rates. These tracings demonstrate the pronounced dynamic constrictor and dilator responses during the periods of changing P_T graded in relation to dP_T/dt both for positive and negative values. They also show the comparatively small static constrictor response in the steady-state period of constant increased P_T. Fig. 2 lower panel illustrates corresponding microvascular resistance responses obtained with the mathematical model. It can be seen that the biological and the simulated curves show great resemblance as regards their fundamental characteristics. Thus the amplitude of the dynamic constrictor and dilator responses and that of the static constrictor response are quite similar and this also applies to the general configuration of the curves except for that of the onset and offset of the dP_T/dt stimulus. The fact that the simulated resistance responses in contrast to the biological ones start momentarily and cease abruptly upon the onset and offset of the dP_T/dt stimulus can, at least mainly, be ascribed to the neglectance in our model of certain biological characteristics viz. time delay and general inertia of the in vivo receptor-effector system and the effects of dynamic

Differentiation of eq (20) gives

$$\dot{p} = \frac{S\dot{A}P + SVP}{2} + \dot{P}_T \tag{24}$$

An expression for $(S\dot{A}P+SVP)/2$ can be obtained by differentiation of eq (23)

Since SVP and R_{prox} can be considered constant during change of P_T (see above) SVP=0 and $\dot{r}_{prox}=0$

Hence

$$\dot{p} = \frac{-2(AP - SVP)\ C\ r_{prox}\ \ r\ \dot{r}}{(r\ + C\ r_{prox})} + \dot{P}_T \tag{25}$$

The starting values for C and C can now be derived by trial and error fitting the configuration of a resistance curve obtained by numerical computer solution of eq (14) (see Results) to a resistance curve (dynamic component) obtained in vivo for one set of arbitrary values of P_T and dP_T/dt (here tested for P_T=30 mmHg dP_T/dt= 3 75 mmHg/s) For this purpose (and for all other tests to be described) the numerical solution of the model according to the Runge-Kutta method (Fox & Mayers 1968) was obtained with the aid of computer ALFA Jumbo LSI 2/20 B This trial and error procedure was performed until there was as close fit as possible between the configuration of the dynamic myogenic resistance curves in vivo and in the model

In this way the starting values for C C C and C were calculated from a given starting value for E From the solution of the mathematical model with these starting values the contractile force developed in the peak of a strong dynamic constrictor response was calculated This force F was obtained from eqs (8) (9) and (10) giving

$$F_p = C\ \sigma_p + C\ \dot{\sigma}_p + C \tag{26}$$

where index p denotes the values for F σ and $\dot{\sigma}$ developed in the peak of the dynamic response The active tension derived from this F value was now compared with corresponding biological data. Data for active myogenic force development in response to a strong dynamic stimulus can be deduced for the single unit vascular smooth muscle of the rat portal vein from observations by Johansson & Mellander (1975) and converted into active tension development by using the data for cross section area given by Johansson (1976) for the same preparation Active peak myogenically induced tension per smooth muscle cross-sectional area thus deduced amounted to about $0\ 4\times10^5$ N/m² (implying that such a myogenic response develops an active tension which is some 20% of the maximal contractile tension (about 2×10 N/m²) that can be elicited by a supramaximal dose of potent vasoconstrictor agents (Johansson 1975))

By continuing the iterative process successively modified values for E and for C_6, C C_3 C_4 and C were obtained until finally the computer solution of eq (14) gave a peak dynamic active tension which approached 0.4×10^5 N/m² and simultaneously a very close fit between the configuration of the myogenic resistance curves in vivo and in the model.

The following final values were thus obtained.

$E=1\times10^4 N\ m^{-2}$ $C = 7\ 0\ \ 10\ N\ m^{-3}$

$C = 1.5\ \ 10^8 Ns\ m^{-1}$ $C_4 = 2.25\times10\ \ m^2$

$C_3 = 3.3\times10\ \ m\ s$ $C = 1.25\ \ 10^{-3} N$

In summary the mathematical model for myogenic microvascular resistance responses to change of transmural pressure can be expressed as follows by combination of eqs. (14) (20) (23) (25) (16) and (26):

$$\dot{r}\left[C_1 + \frac{C\ p}{\alpha h}\left(1 - \frac{r}{h}\ \frac{dh}{dr}\right)\right] + $$

$$+ r\left(C\ h + \frac{C\ p}{\alpha h} + \frac{C_4 \dot{p}}{\alpha h} - pL\right) = C\ hr\ - C \quad \text{eq (1}$$

where

$$p = \frac{C\,(AP + SVP)\left(\frac{r_{prox}}{r}\right) + 2SVP}{2\left[1 + C\left(\frac{r_{prox}}{r}\right)^4\right]} + P_T, \quad \text{eqs. (20)+(23)}$$

and

$$\dot{p} = \frac{-2C\,(AP - SVP)\,r_{prox}\ \ r}{(r\ + C\ r_{prox})^2}\ \dot{r} + \dot{P}_T, \quad \text{eq (25).}$$

$$R_{micro} = C_{micro}\ \frac{l}{r} \quad \text{eq (16).}$$

$$F_p = C_4\,\sigma_p + C_3\,\dot{\sigma}_p + C \quad \text{eq (26).}$$

The first equation (14) after insertion of the expressions for p [eq (20)] and $\dot{p}$ [eq (23)] is the differential equation for the microvascular radius (r) change in response to experimental variation of transmural pressure (p) produced by alteration of P_T (in change of extravascular pressure) To permit comparison with microvascular resistance responses in vivo the radius change in the mathematical model is converted into resistance change with the aid of eq (16) The maximal myogenically developed force finally was calculated according to eq (26) Below are summarized the collected data for the different numerical constants in the model

$L=10^{-4}$ m $=13\times10^{-6}$ m $\alpha=0\ 5$ $C=7\ 0\times10^6$ N m^{-3} $C=1.5\times10^8$ Ns m $C=2.25\times10^{-6}$ m^2 $C=3\ 3\times10^{-5}$ m^{2}s $C=1\ 25\times10^{-4}$ N $C=2.7\times10^{-4}$ AP=105 mmHg $(1\ 4\times10^4$ N m$^{-2})$ SVP=15 mmHg $(2\times10^3$ N m$)$ $r_{prox}=29\times10^{-6}$ m and $C_{micro}=1.5\times10$ PRU m

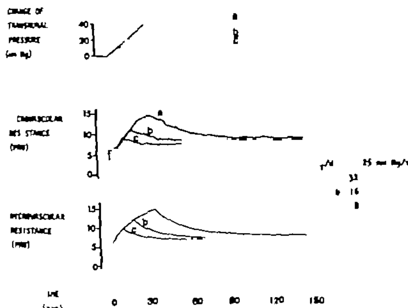


Fig. 4. Comparison between myogenic microvascular resistance responses obtained with the mathematical model and corresponding in vivo responses evoked by graded increases in vascular transmural pressure P_T by 10, 20, and 40 mmHg above control applied at constant rate of 1.25 mmHg/s. The resistance recordings are reproduced on top of each other. Note in particular that the active dynamic response increases continually throughout the whole period of dP_T/dt stimulation. Also note the graded static response.

wall tension (σ), its rate of change ($\dot{\sigma}$) and internal vessel radius ( ) during change of transmural pressure. If so, the model might help to explain the causal interrelationship between myogenic stimulus and vascular effector response.

Fig. 5 shows how the mentioned three parameters (and) and R_{micro} vary in the model during a P_T increase of 30 mmHg applied at a rate of 2.5 mmHg/s, during the subsequent steady-state phase of constant increased P and during decrease in P back to the control level at the same given rate. Upon the onset of the dP/dt stimulus rate of wall tension change $\dot{\sigma}$ [defined by eq. (13)], reaches momentarily a high positive value creating the main driving force ($C_6\dot{\sigma}$) for the dynamic constrictor response [eq. (12)]. The resulting decrease in internal radius ( ) in turn leading to a negative value of $\dot{r}$ and to some increase in wall thickness h [eq. (1)] counteracts according to eq. (13) this $\dot{\sigma}$ increase. On the other hand the concomitant rise of intravascular transmural pressure (p) due to constriction induced increase in SAP [eqs. (23) or (25)] counteracts this decrease of $\dot{\sigma}$ [see eq. (13)]. In spite of the gradual decline of σ the dynamic force is strong enough to cause a continuing increase in R_{micro} throughout the period of increasing P (Figs. 4 and 5). This latter effect can in part be explained by an increasing gain ($\Delta R_{micro}/\Delta r = K$ where K is a constant) implicit in the inverse forth power relationship between R_{micro} and r according to Poiseuille's law (see Fig. 5 panels for R_{micro} and internal radius variation). This increase in gain can also explain the findings obtained with the present model and also in vivo (see Grände 1979) that the dynamic response to a standardized transmural pressure stimulus increases with increasing control resistance per se.

Note that the type of positive feed-back inherent in the described p increase resulting from constriction [first term in eq. (13)] is counteracted by effects of Laplace's law viz. by a concomitant r decrease [second term in eq. (13)] which effectively helps to avoid instability in myogenic control.

Upon cessation of the dP_T/dt stimulus during constant increased P (Fig. 5) $\dot{\sigma}$ falls abruptly and hence the dynamic constrictor response no longer

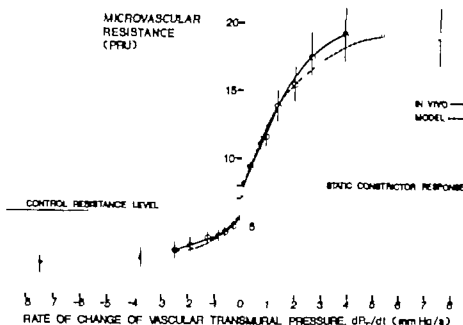


Fig 3 Comparison between dynamic myogenic microvascular constrictor and dilator responses obtained with the mathematical model (dashed curve) and corresponding average responses in vivo (solid curve) evoked by a given vascular transmural pressure (P_T) change by 30 mmHg applied at different rates dP_T/dt in the range from −7.5 to +7.5 mmHg/s The average static constrictor response is also shown. Note the good agreement between model and in vivo results (the latter reproduced from Grande & Mellander 1978 by permission)

myogenic pacemaker spike afterdischarge (see further Discussion) Such differences might also explain the absence in the model of the damped gross oscillations noted in vivo especially when the resistance responses during recovery return to control steady state

Fig 2 further shows that the dynamic constrictor and dilator responses in vivo as well as in the model were distinctly graded in relation to the applied dynamic stimulus dP_T/dt These characteristics are illustrated in greater detail in Fig 3 which depicts average in vivo results from Grände et al 1978 (solid curve) and the computer results (dashed curve) in terms of the dynamic microvascular constrictor and dilator responses to dP_T/dt stimuli over the entire range from −7 5 to +7.5 mmHg/s It can be seen that the mathematical model quite accurately reflects the in vivo responses (see Discussion)

Fig. 4 illustrates on a comparative basis the dynamic resistance responses to a given dP_T/dt stimulus (1 25 mmHg/s) of different duration (8 16 and 32 s) and the static steady-state responses to three different P_T stimuli (10 20 and 40 mmHg) Note in particular the phenomenon that the active dynamic response increases continually through the whole period of dynamic (dP_T/dt) stimulation an effect which will be further considered below

The presented data thus show not only for t trimming P_T and dP_T/dt value (30 mmHg 3 mmHg/s) but also for the whole wide range of te good agreement between in vivo and comput results This seems to warrant the conclusion th the present model quite adequately can descri myogenic microvascular resistance responses transmural pressure stimuli This mathematical a proach thus supports the myogenic hypothesis general and makes it likely that myogenic reactio are triggered by changes in wall tension It furth strengthens the concept of an important rate-sens tivity in myogenic microvascular control a co clusion also corroborated by the finding that elim nation in the model of the dynamic force C_d i eq (12) above abolished all presented resemblanc to biology

From such considerations it appears that th model in addition can permit determination c some other variables in the myogenic microvascula control system which are difficult (or impossible to assess by conventional in vivo observations e g

g a new steady state with a small remaining increase in R_{arm} above control This is the static myogenic response and is caused by the σ increase raising the force $C_{\sigma}\sigma$ [eq (14)].

Although the static response starts to develop at the onset of the P_T increase it rises further during the period of constant increased P_T due to the continuing increase of σ (see Fig. 5) which is an effect of Laplace s law [eq (3)].

At the start of the P_T decrease back to control (negative dP_T/dt stimulus) $\dot{\sigma}$ falls momentarily to a negative value creating a strong inhibitory dynamic force ($C_{\dot{\sigma}}$) which initiates active dilation The induced decrease in SAP makes σ still more negative reinforcing the dilation (positive feed-back) The latter effect, however is to some extent counteracted by the increasing r [positive $\dot{r}$ eq (13)], which stabilizes the reaction.

At the moment P_T reaches the control level (offset of negative dP_T/dt) the inhibitory dynamic force ($C_{\dot{\sigma}}$) is almost eliminated, and therefore the normal vascular contractile force can gradually restore microvascular tone Thus constriction is however somewhat counteracted by effects of Laplace s law [negative in eq (13)] giving negative in spite of the induced increase in SAP

The data in Fig. 5 can also be used to calculate the active myogenic forces developed in the microvascular smooth muscle in response to a change in overall transmural pressure According to eq (26) the myogenic force is represented by $F = C_{\dot{\sigma}}\sigma + C_{\sigma}\sigma + C$ The myogenic force (F) is developed at the effect of the positive dynamic stimulus. In this situation σ and σ (calculated for the fraction of vessel wall containing active tension) were $_5\times10^4$ N/m² and 6×10^4 N/m²s, respectively With the obtained numerical values for C C and C F was calculated to be 8.4×10^{-4} N which corresponds to an active wall tension of 3.6×10^4 N/m²

DISCUSSION

Myogenic reactions to passive stretch and shortening in vascular smooth muscle can in view of the recent in vitro studies mentioned in Introduction be explained by positive and negative chronotropic effects on pacemaker cells and consequent changes in the contractile state of the effectors Later studies (see Introduction) support the view that such a mechanism is operating in the microvessels in vivo as well The situation in vivo however is much complicated by the effects of Poiseuille s and Laplace s laws and of wall/lumen variations on wall tension and the tendency of a positive feed-back inherent in the interrelation between myogenic vasoconstriction/dilation and intravascular pressure The latter considerations have even led some authors (e g. Guyton 1964) to more or less reject the myogenic theory first, because of postulated intolerable instability caused by positi e feed-back in myogenic control and second because of proposed self-limitation in the system due to abolition of stimulus (decreased sensor length) once the myogenic constriction has developed Even if some arguments against such reasoning were provided by the direct observations of myogenic control in vivo and in vitro (see Folkow 1964 Mellander & Johansson 1968 Johnson 1977 and the studies mentioned in the Introduction) there is still a demand to define in more precise terms the events predicted in myogenic control

The use of mathematical models for description of complex biological systems as the myogenic control system can greatly help to elucidate the physical nature of the postulated processes in this control system and its basic characteristics. Such an attempt was made by Kock in 1964 but his models described only steady-state situations without taking into account the dynamics in myogenic control

An attempt was made in this study to design a model which from a functional point of view resembled the in vivo situation reasonably well Thus seemingly reasonable data for morphological and structural quantities were derived from the current biological literature and our model for myogenic reactions to change in transmural pressure also took into account not only the static steady-state component but also the recently described important rate dependent component in myogenic control. Yet, in this model like in any other mathematical approach to complex biological systems, it was necessary to make certain simplifications some of which deserve further comments

An obvious simplification in the present model is the neglectance of the effects of general inertia in the biological system. Phasic change of transmural pressure in vivo leads to a gradually developing resistance response due to delay in pressure transmission and in the receptor-effector reaction etc Further there is a maintenance of the rate-dependent response in vivo for some time after

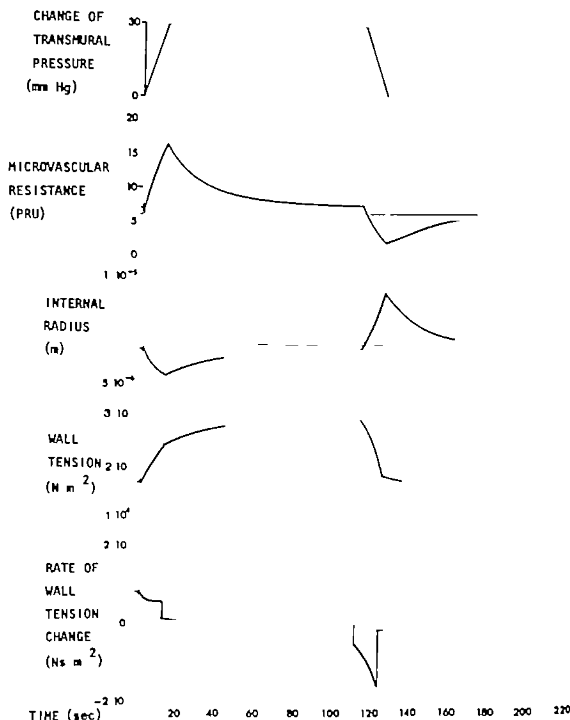


Fig 5 Variation of microvascular resistance (R_{micro}) internal radius ( ) wall tension (σ) and its rate of change ($\dot{\sigma}$) as obtained with the model during an increase in P_T by 30 mmHg applied at the rate of 2.5 mmHg/s, during the following period of constant increased P_T and during subsequent decrease in P_T back to the control level at the rate of −2.5 mmHg/s Note in particular the marked step increase/decrease of σ at the onset of the positive/negative dP_T/dt stimulus creating the main driving force for the obtained active dynamic constrictor/dilator responses (Further explanation see text.)

can be maintained thus resulting in an increase in r and a decrease in R_{micro}. This relaxation of the vessels occurs gradually due to the effect of the wall viscosity [C_1 $\dot{r}$ eq (17)] and to some extent to the small still positive towards zero decaying σ This positive σ is the result of an increasing r [Laplace's law eq (13)] however counteracted by the negative $\dot{p}$ in turn caused by the relaxation induced fall of SAP [eqs (23) or (25)]. During the later period of constant increased P_T (Fig. 5) $\dot{\sigma}$ reaches zero gr

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the cessation of the dynamic stimulus most likely caused by an electric afterdischarge in the pace maker' as demonstrated in vitro (Johansson & Mellander 1975) Neglectance of these phenomena most likely explains why the model curves start and cease abruptly upon onset and offset of the dynamic stimulus and hence in these respects deviate from the more smooth on onset upwards concave bio-logical' configuration of resistance curves in vivo (see Figs 2 and 3)

Our finding of a very large mean radius (13 μm) of the microvessel upon virtual elimination of passive and active forces (see above) when accomplished by decrease in arterial inflow pressure to a very low level (<10 mmHg) and at zero venous pressure on the paralysed vascular bed is in agreement with vital microscopy findings that arteriolar vessel closure does not occur at zero transmural pressure (e g Eriksson & Myrhage 1972 Gore 1972) The net passive elastic force (F_E) according to eq (4) is thus operating in the outward direction tending to increase vessel radius within the normal physiological range of radius (r<13 μm) This finding is not compatible with the concept of critical closure (see Burton 1962) When the microvessel radius is larger than 13 μm the net elastic force will however operate in the opposite direction Had we assumed F_E to operate in the inward direction tending to decrease vessel radius the mathematical model with reasonable values for E gave a solution which bore no resemblance to biology at all a result which further strengthens the view that the net elastic force in the microvessel in fact is directed outwards. The absence of passive elastic recoil under the mentioned experimental conditions might be explained by the characteristics of a relatively thickwalled microvessel and by possible anchoring of its exterior to the surrounding tissue an opinion which seems compatible with the tunnel in a gel behaviour for capillaries described by Fung (1966)

The present model predicted that the elastic force F_E under the described conditions is directed outwards tending to increase vessel radius Its magnitude which would be difficult to determine in vivo was in the order of 1×10^4 N/m^2 Elasticity in vivo has been determined in microvessels during passive stress by Gore (1977) Under these circumstances the vessel radius is larger than r_0 why the elastic force is directed inwards and was then in the range from $3-12\times10^4$ N/m^2 depending on the degree of passive distension It is evident that values for vessel elasticity determined by the two different methods and under quite differe[nt] conditions are not directly comparable

The fact that wall thickness (h) and thus al[so] wall/lumen vary with change of radius in thic[k] walled vessels was taken into account in this mod[el] (see eq 1) but the profile of tension (σ) across th[e] wall was assumed constant Therefore the mod[el] refers to mean wall tension but this simplificatio[n] was calculated to cause only a very small error

The active myogenic forces in this model wer[e] assumed to be related to σ and $\dot{\sigma}$ (eqs. (8) and (9) and hence independent of actual muscle length. This neglectance of possible shift along length tension curve might have led to some over-estima[tion] of the constrictor and dilator responses in th[e] model to judge from length-tension data for th[e] portal vein single unit muscle (cf Johansson 1975 and for large arterioles (Mulvany & Warshaw 1979) The relatively small range of radius variation tested in this model implies however that even for the worst case the inherent error must be quite small a conclusion supported by the presented similarity between in vivo and model resistance curves

From these considerations it seems fair to conclude that our model is reasonably valid for description of myogenic microvascular resistance responses to change of transmural pressure The presented results are compatible with the concept of an important rate-sensitivity in myogenic vascular control system and they strongly suggest that dynamic myogenic responses are triggered by and related to changes of wall tension The results demonstrate that the tendency of positive feed-back in myogenic control does not lead to instability as proposed by some authors The model also provided data for the behaviour of some variables which are difficult to assess by conventional in vivo techniques such as Young s modulus of elasticity wall tension its rate of change internal vessel radius and viscous properties of the arterial micro-vessels Furthermore the model could offer a means to define more precisely the role of certain physical factors (e g effects of Laplace s and Poiseuille s laws) in vascular blood flow regulation.

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Capillary permeability in cat choroid studied with the single injection technique (II)

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TÖRNQUIST P Capillary permeability in cat choroid, studied with the single injection technique (II) Acta Physiol Scand 1979 106. 425–430. Received 31 Jan. 1979 ISSN 0001-6772. Institute of Physiology and Medical Biophysics and Department of Ophthalmology University Hospital University of Uppsala, Sweden.

Transcapillary movements of [illegible]Na, [illegible]Cr EDTA and [illegible]I-myoglobi were studied i the cat choroid by means of the single injection technique using labelled albumin as the reference tracer By an external shunting of blood, recirculation of tracer was delayed and complete venous outflow curves became available for analysis. The initial extractions observed were 0.66, 0.35 and 0 08 respectively The extractions decreased with time Within 20–25 s the extracted fractions of the tracers had almost completely returned to the blood. The results indicate high permeability coefficients and small extravascular volumes of distribution The retinal pigment epithelium seems to have very low permeability to all three substances. Extraction of tritiated water at 20–25 was about 0.30 probably due to rapid penetration of labelled water into the retina. Evidence for stereo-specific transport system for glucose in the retinal pigment epithelium was found in experiments with labelled D-glucose and 3-O-methyl-D-glucose The total amount of glucose delivered by the choroid was calculated from measurements of the a- differences for unlabelled glucose.

Key ords Single injection technique choroidal capillary permeability glucose

In cats as in primates the retina is nourished by two different vascular systems, the retinal and the choroidal vessels. The choroidal vessels supply the outer parts of the retina The capillaries are solely located in a single plane the choriocapillaris, which is separated from the photoreceptors by Bruch s membrane and the retinal pigment epithelium The ultrastructure of the choriocapillaris is similar to that in the capillaries of the intestinal mucosa and the choroid plexus e the visceral type. Thus the capillary wall is thin with large fenestrations covered by thin membrane Physiological studies of the choriocapillaris have shown a high permeability to fluorescein (Grayson & Laties 1971) even macromolecules pass out at low rate (Bill 1968) The fluorescein leaving the choriocapillaris however does not pass the retinal pigment epithelium which makes up the epithelial part of the blood-retinal barrier Electron microscopic studies with horse radish peroxidase have shown that intercellular diffusion is restricted by the tight junctions between adjacent cells (Shiose 1970) The retinal pigment epithelium thus seems to effectively separate the retina from the blood with regard to water-soluble substances. To fulfil the metabolic needs of the retina the barrier must have mechanisms for active transport and/or facilitated diffusion of nutrients and metabolic end products.

The vascular anatomy of the cat eye permits collection of choroidal venous blood. Thus the single injection technique (Crone 1970) can be applied to estimate the permeability of the choriocapillaris. Preliminary experiments have shown a very high permeability to sodium (Törnquist 1977) The purposes of the present study were:

1 To characterize the permeability properties of the fenestrated capillary wall with regard to low molecular weight water-soluble substances by applying the single injection technique to the cat choroid

2. To calculate the amount of glucose delivered by the choroidal circulation to the retina by measur

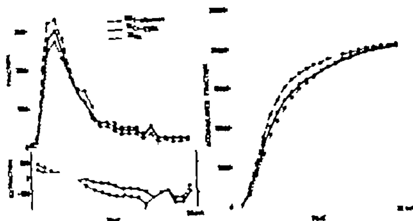


Fig. 1 ( ) Upper left: the venous outflow curve from a typical experiment. (*b*) Lower left: the fractional extraction as function of time (*E* vs. time). ( ) Right: the integrated venous outflow curve.

...was pipetted into 10 ml of Instagel (Packard Instrument Company Inc., Downer's Grove, Illinois, USA) and radioactivity was determined in scintillation counter. An external standard was used for quenching correction. The routines were the same as for gamma spectrometry. All results were then analyzed with a 24-K computer.

Tissue extraction studies

The glucose concentration of arterial and venous blood was determined with the glucose oxidase method (Glox, Kabi, Sweden). Three to 5 blood samples were taken and the experiments were accepted only if the values agreed within 5% from the mean. The means of the samples were used to calculate the a-v difference. By aid of the single injection technique the tissue extraction of ¹⁴C labelled glucose (5 cats) and 3-O-methyl-D-glucose (6 cats) was measured with ³H labelled L-glucose as the reference. Tracer amounts of labelled glucose (obtained from NEN ...) were dissolved in plasma. The procedure of injection, venous blood sampling and further treatment of the samples was the same as in the expts. with ³HOH and ²²Na.

Statistics

The following mean and S. E. are given. Student' t-test was used to determine the statistical significance.

RESULTS

The experiments were performed at a blood pressure of about 150 cmH$_2$O and an intraocular pressure of about 20 cmH$_2$O

Choroidal permeability to ²²Na, ¹⁴C EDTA and ¹²⁵I-myoglobin

Fig. 1*a* shows a typical venous outflow curve from the experiment in which the loop was used. The concentration of each indicator in every venous blood sample is expressed as a fraction of its concentration in the mixture that was injected i.a. and is plotted against time

Fig. 1*b* shows the fractional extraction as a function of time. The initial extraction value E_{max} for a test substance was calculated from the first sample in which the reference substance appeared. The activity in this sample was at least 2–3 times that in the samples collected before the injection. The maximal extraction was found in the first sample E_{max}, following which the values fell with time. A negative value indicates a net flux from the tissue to the blood (back-diffusion). The negative slope of the *E* vs. time curve was a general finding.

In Fig. 1*c* the venous outflow curves are integrated. It is then possible to calculate the accumulated loss of test substance $E_{(net)}$, after 20–25 s, within the recirculation-free period

The extractions obtained in the whole material are shown in Table 1. For each animal 2 to 3 injections were given and the mean values for the individual animals were averaged. In the expts. with the loop significant E_{max} values were obtained for both ²²Na and ⁵¹Cr EDTA ($P<0.001$) and also for ¹²⁵I-myoglobin ($P<0.02$). Without the loop the corresponding values tended to be higher. The only $E_{(net)}$ that significantly differed from zero was the value for ²²Na ($P<0.02$).

From the expts. with two test substances simultaneously injected, the $P_{Cr\text{-}EDTA}/P_{Na}$ and the $P_{myoglobin}/P_{Na}$ ratio were calculated from the E_{max} val-

ing the a-v difference and using earlier data on choroidal blood flow

3 To find out whether retinal glucose uptake from the choroidal circulation has a stereo-specific preference by comparing the tissue extraction of intraarterially injected D and L-glucose

METHODS

The single injection technique theory

Transcapillary movements of molecules can be studied by injecting intraarterially a mixture of two indicators and taking blood samples from the vein draining the investigated tissue after one passage of the indicators through the capillary system (Crone 1963*b*) One indicator (the reference substance) is selected such as to remain intravascular Albumin (MW=67 000) fulfils this criterion for single injection experiments in the cat choroid (Törnquist 1977) The concentrations of tracers are measured in the venous blood and normalized with respect to those in the injectate The concentration of the reference substance in the individual sample is a measure of the degree of intravascular dilution If the relative concentration of the test substance is less than the relative concentration of the reference substance this indicates an extravascular loss. The fractional extraction at any instant is defined as the difference in concentration between the reference and the test substance divided by the concentration of the reference substance The extraction of a hydrophilic diffusible substance measured by this technique is related to the permeability of the vessel according to the formula

$$PS = -Q \ln (1-E)$$

where P is the permeability coefficient (cm/s) S the available capillary surface area (cm²/g) Q the flow of tracer carrying fluid (ml/g s) and E the measured extraction. The derivation of the formula rests on the assumption that there is no back-diffusion of the test substance from the tissue to the blood. However with a limited extravascular space back-diffusion will occur Then the initial extraction value E_{in} calculated from the venous blood sample in which the reference substance first appears is least influenced by back-diffusion

With two simultaneously injected test substances the ratio of the permeability coefficients of the substances is related to the extractions by the equation $P_1/P_2 = \ln(1-E_1)/\ln(1-E_2)$ (Crone 1963*a*) Thus S and Q are eliminated if it can be assumed that they are the same for the two test substances The P_1/P_2 ratio can be compared to the ratio of the free diffusion coefficients for the same substances D_1/D_2 Restricted diffusion through pores in the capillary membrane causes a disproportionality between these ratios

Preparation

Cats of both sexes weighing about 3 kg were used Anesthesia was induced with chloroform and maintained with chloralose and if necessary urethane Body temperature was kept normal with a heating pad After tr cheotomy artificial ventilation was adjusted to give arterial P_{CO_2} of about 4 kPa (30 mmHg) and a pH of 7 Heparin was given to prevent clotting. One brachial arter was cannulated and connected to a pressure transducer to record arterial blood pressure. The anterior chamber w cannulated with a steel cannula to measure the intraocul pressure In 20 animals recirculation of blood containi the indicators was prevented for some time with an exte nal shunting of blood from the abdominal aorta via polyethylene catheter to the carotid artery the proxim part of which was ligated The catheter was prefilled wi either Rheomacrodex® (Pharmacia, Uppsala, Sweden) c saline The fluid in the catheter was kept at body-tempera ture with a water bath. Blood pressure was measured i the carotid artery distal to the tip of the external loop. Th blood flow in the external loop could be controlled with roller pump and was adjusted to give a pressure in th carotid artery of about 1.5 kPa (10–15 mmHg) above th in the contralateral brachial artery In order to reduc interference by the contralateral circulation with the capi lary bed of the investigated eye. The arterial injection were made with a push-pull system into the catheter nea its entrance in the carotid artery Three cats were pre pared without the external shunt In these animals th arterial injections were made into the carotid artery via cannulated thyroidal artery In all cats the temporal part o the conjunctiva was removed in order to reach the intra scleral venous plexus where a suitable vein was cannu lated with a polyethylene catheter The catheter was ad vanced into a choroidal vein (see Bill 1963).

Permeability studies

In 21 cats albumin was used as the reference substance I was prepared from rabbit plasma with conventional tech nique The purity was checked with disc electrophoresis The albumin was labelled with ^{125}I or ^{131}I and purified from free iodide the day the experiment was performed. Three different test substances were used myoglobin (MW= 17 500) (Serva Heidelberg Germany) labelled with ^{131}I and purified as was the albumin, ^{51}Cr labelled EDTA (MW=362) (Hoechst, Frankfurt (Main) Germany) and sodium (^{22}NaCl from NEN USA) A mixture of reference and test substance was dissolved in saline In 11 expts two test substances were used simultaneously In 2 expts in which ^{22}Na was used as reference substance and HOH (obtained from AB Atomenergi Studsvik Sweden) as test substance the tracers were mixed with blood Samples were taken from the injectate A volume of about 0.2–0.3 ml was injected intraarterially over less than 2 s Venous blood samples were collected during the time free from recirculation. This time varied between 20 and 40 s and was experimentally determined by measuring the transit time for Evans blue through the external loop giving the actual flow rate in the shunt. The blood samples were collected on a rotating filter paper at a rate of one to three samples per s and analyzed for radioactivity by gamma spectrometry Each sample was counted twice The counting time was chosen to obtain more than 1 000 counts after background subtraction In the experiments with ^{3}HOH 50 µl samples of venous blood were collected in capillary tubes. After immediate centrifugation 25 µl of

...of the capillaries, since the capillaries form a ...et in a single plane at the surface of the ...d. With these values for Q and S inserted in formula $P = -(Q/S) \ln (1-E)$ the P for Na and ...r EDTA are about 180 and 70×10⁻ cm/s respectively. These values are about 50 and 80 times the ...ues reported for the capillaris in skeletal muscle ...and 0.[illegible]×10⁻⁵ cm/s respectively (Trap-Jensen & Lassen 1970). Thus the results indicate a very high permeability to small hydrophilic substances such as amino acids and glucose in the choriocapillaris. Even rather large molecules such as myoglobin seem to diffuse through the capillary wall at a high rate.

A similarity between the ratio of the permeability coefficients of two tracers P_i/P_k, and the ratio of their free diffusion coefficients D_i/D_k has been used as an argument against restricted diffusion (Crone 1963a). From the experiments with two simultaneously used test substances in the present study the obtained ratio $P_{Cr-EDTA}/P_{Na}$, 0.56 is higher than the corresponding ratio of their free diffusion coefficients, indicating an underestimate of P_{Na} as compared to $P_{Cr-EDTA}$. If the E_{max} for ²²Na is an underestimate in relation to less diffusible molecules than a $P_{myoglobin}/P_{Na}$ ratio of 0.08, which is identical to the $D_{myoglobin}/D_{Na}$ ratio does not prove free diffusion of myoglobin but rather suggests a tendency to restricted diffusion of myoglobin. The results thus suggest that back-diffusion caused underestimated PS products and affected the more diffusible tracer to a greater extent.

By introducing the external loop in the experiments a great part of the E vs. time curves became available for analysis. To exclude the possibility that the more extensive surgery and the loop caused an increased vascular permeability by releasing some vasoactive substance control expts. were performed, in which the i.a. injections were made in a ciliary artery. The results obtained do not support the suspicion of increased permeability since the E_{max} values in these expts. in fact were higher than in those with the loop (Table 1). A possible explanation for this somewhat surprising result is a more pronounced Taylor diffusion due to longer catheters used for injections and blood sampling in the expts. without the loop (see Renkin 19..).

The E_{mean} calculated at the end of the recirculation free period in the experiments with the loop showed no significant loss of ⁵¹Cr EDTA or ¹²⁵I-myoglobin. This result indicates that the molecules that had passed out into the tissue had returned. This complete return must be due to a limited and small extravascular distribution volume. The loss of a small fraction, E_{mean}, of ²²Na may be the result of some ²²Na penetrating into cells and ²²Na transported into the retina by the retinal pigment epithelium (Miller & Steinberg 1977) or ²²Na entering the retina through the tight junctions of the retinal pigment epithelium. At the time of complete return of ⁵¹Cr EDTA and ¹²⁵I-myoglobin a considerable fraction of ³HOH was still extracted suggesting that HOH diffused into the retina. ³HOH can use transepithelial cell pathways (Renkin 1978) in other tissues and is unlikely to respect the blood-retinal barrier. However the net flux of water is probably from the retina to the choroid due to the higher oncotic pressure in the choroid (Bill 1975).

The fenestrated capillaries of the choroid do not appear to have much of a barrier function with regard to diffusion of small water-soluble substances as indicated in this study. Consequently the concentration of glucose in the interstitial fluid that surrounds of basal portion of the retinal pigment epithelium can be expected to be very similar to that of plasma. Further passive diffusion of glucose into the retina is unlikely since the retinal pigment epithelium seems to provide a considerable diffusion barrier even to small molecules. However the expts. with labelled glucose show a significant loss of D-glucose when L-glucose was used as reference substance. As D-glucose and L-glucose have similar diffusions properties this result indicates a stereospecific transport system presumably in the retinal pigment epithelium. The results obtained with 3-O-methyl-D-glucose also indicate a carrier mediated transport, but whether these two monosaccharides share the same carrier as has been reported for the brain (Betz & Gilboe 1974) or not has not been investigated in this study.

There was a net glucose loss of 0.21 mmol/l from blood passing through the choriocapillaris. Assuming that the cannulated vein drains relatively pure choroidal blood and the choroidal blood flow is 734 mg/min (Alm & Bill 1972) the mean value for the total amount of glucose lost through the choroid is 154 nmol/min. Alm & Bill (1972) estimated the amount of oxygen extracted from the choriocapillaris to be 5.6 µl/min in cats (250 nmol/min). If all oxygen is used for glucose catabolism the amount of glucose completely oxidized to CO_2 and water is

Table 1 *Extracted fractions* ± S E (n)

	$E_{(\max)}$ Without loop	$E_{(\max)}$ With loop	$E_{(\mathrm{rec})}$
^{22}Na	0 93±0 003 (3)	0 66±0 06 (18)	0 03±0.01 (12)
^{51}Cr EDTA	0.59±0 07 (3)	0.35±0 04 (7)	0 01±0 01 (5)
^{125}I myoglobin	0 13±0 13 (2)	0 08±0 02 (6)	−0 003±0 08 (3)

ues and were found to be 0 56±0 04 ($n=6$) and 0 08±0 01 ($n=5$) respectively The corresponding figures for the ratio of the same substances free diffusion coefficients are 0.39 and 0 08 $P_{Cr-EDTA}/P_{Na}$ differed significantly from $D_{Cr\ EDTA}/D_{N}$ ($P<0\ 01$)

Choroidal permeability to ^{3}HOH

Seven injections were performed on 2 animals Because of the analytic procedures relatively large venous blood volumes were required resulting in poor time resolution Thus a relevant $E_{(\max)}$ value could not be determined After the recirculation free period 20–25 s the $E_{(\mathrm{rec})}$ for ^{3}HOH was 0 30±0 05 ($n=7$) ($P<0\ 005$) using ^{22}Na as reference

Glucose extraction studies

The mean arterial glucose concentration was 11 45±0 84 mmol/l and the a v difference was 0 21±0 10 ($n=19$) ($P<0\ 05$) The a-v difference represents the net flux of glucose leaving the vascular compartment and corresponds to 0 07 of the arterial glucose concentration The $E_{(\mathrm{rec})}$ of D-glucose and 3-O-methyl-D-glucose at 20–25 s were 0 14±0 03 ($n=5$) ($P<0\ 02$) and 0 05±0 01 ($n=6$) ($P<0\ 02$) respectively when compared to L-glucose

DISCUSSION

If the reference substance remains in the vascular compartment as has been shown for albumin in the choroid (Törnquist 1977) a separation of the venous outflow curves such as seen in the present expts could be due to an intravascular separation of the tracers or a transcapillary exit and subsequent reentry of the test substance Intravascular separation could be due to an interlaminar diffusion The magnitude of this effect is positively correlated to the free diffusion coefficients (Taylor 1953) and the effect is to cause apparent positive extractions of low molecular weight substances as compared to high molecular weight material initially and in [the] end of the E vs time curve (Lassen & Crone 197[0]) By using the external loop in the present exp[ts] relatively complete venous outflow curves w[ere] obtained No late positive extractions were se[en] Thus Taylor diffusion can only have had a sm[all] influence on the results obtained in these expts

If the intravascular separation is small then [the] E values reflect a net tracer flux over the capill[ary] membrane However the formula $PS=-Q\ln(1-E)$ is valid only when E is a measure of unidirectiona[l] efflux of tracer In the present expts a commo[n] feature of the E vs time curve was the negativ[e] slope The most probable explanation for th[is] phenomenon is early back-diffusion of tracer fro[m] the tissue to the blood This interpretation is su[p]ported by the finding that after the recirculation fr[ee] period practically all of the tracer had returned [to] the blood This rapid back-diffusion indicates [a] highly permeable capillary membrane and a rel[a]tively small extravascular distribution volume [as] could be expected from the morphology of th[e] choroid In an attempt to minimize the interferenc[e] of back-diffusion $E_{(\max)}$ was calculated from the firs[t] sample that contained significant amounts of th[e] reference substance However the calculated $E_{(\max)}$ could still be influenced by back diffusion causin[g] too low values

The initial venous sample comes from the ves[sels] with the shortest transit times The $E_{(\max)}$ thus gives information about regions with the highes[t] linear blood flow rate Regions with lower flow rates may have even higher extractions Thus there are two reasons to suspect that the $E_{(\max)}$ underestimates the average tissue extraction

From blood flow measurements in the cat choroid (Alm & Bill 1973) the rate of plasma flow can be calculated about 7 3 µl/s for the whole tissue In flat mount preparations the total surface area of the cat choroid was measured about 4 5 cm² This area can be assumed to fairly well agree with the surface

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Effects of duct ligation on choline acetyltransferase activity in salivary glands of rats

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Duct ligation was found to cause decrease in the weights of submaxillary and parotid glands examined 3 weeks postoperatively Choline acetyltransferase activity in ligated glands was compared with that in unligated contralateral glands The enzyme activity was also measured in the glands from both sides of unoperated control animals. Interference in the assay of choline acetyltransferase by other acetylated compounds was avoided by introducing suitable control incubations. Ligated submaxillary glands showed a small decrease in the activity of choline acetyltransferase both when compared with contralateral glands and with glands of control rats. In parotid glands the enzyme activity was found to be lower only when ligated and contralateral glands were compared. Structural changes in the nerves and reduced traffic of impulses in them may have to be considered as explanations for the reduction in enzyme activity in duct-ligated glands

Key words Choline acetyltransferase duct ligation, salivary gland atrophy

Duct ligation of a salivary gland causes secretory cells to atrophy (Tamarin 1967 Donath Hirsch-Hoffmann & Seifert 1973 Harrison & Garrett 1976*a*) and the activity of respiratory enzymes to decrease (Junqueira 1951 Harrison & Garrett 1976*b*) The secretory capacity of such a gland is greatly reduced both in response to secretagogue drugs (Ohlin & Perec 1967 Emmelin Garrett & Ohlin 1974) and to nerve stimulation (Emmelin et al 1974). Choline acetyltransferase the acetylcholine synthesizing enzyme, is confined to the cholinergic nerves in salivary glands (see Ekström 1978) and in the present study the effect of duct ligation on this enzyme was investigated in the parotid and submaxillary glands of rats. The activity of choline acetyltransferase was estimated by a radiochemical method, however difficulties were encountered in identifying acetylcholine and these were solved by using additional controls in the assay procedure In addition the effect of parasympathetic denervation on parotid glands was studied.

METHODS

Animals and surgical procedure

35 male adult rats, weighing about 350 g, of Sprague Dawley strain bred at the Department of Physiology Lund, were used Operations were performed under ether anaesthesia and with the aid of a dissecting microscope. The right duct of either the parotid or submaxillary gland was ligated with fine silk thread Care was taken to ligate the submaxillary duct as close to its entrance into the mouth as possible in order to avoid damage to the parasympathetic nerves of the gland which travel along the posterior part of the duct towards the gland (see Harrison & Garrett 1972). Parasympathetic denervation of the right parotid gland was achieved by avulsing the auriculotemporal nerve at the point of its emergence from the base of the skull

Twenty-one days after ligation or 6 days after denervation the animals were killed by ether inhalation. Parotid and submaxillary glands were removed, the sublingual glands were discarded. The glands were then cleaned weighed and stored at −20°C. Gland of litter mates served as controls to the animals with ligated ducts together with the contralateral unligated gland. In animals with denervated glands the contralateral gland served as control.

42 nmol/mm This value is considerably lower than the mean value for glucose extraction obtained in the present study but well within the limits of the 95% confidence interval

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acetylcholine. These conditions were not necessarily optimal for carnitine acetyltransferase but gave good values for pure enzyme (obtained from the Boehringer Corporation, London).

The capacity of the glands to form acetylcarnitine was 4–8 times their capacity to form acetylcholine. It is thus possible that in the choline acetyltransferase assay carnitine acetyltransferase present in tissue homogenates [illegible] endogenous carnitine and [*methyl*-^{3}H] acetyl-CoA to form [*methyl*-^{3}H] acetylcarnitine but since this is not hydrolysed by acetylcholinesterase any activity is accounted for by the subtraction of the control value. The possibility remained, however that carnitine acetyltransferase could synthesize [*methyl*-^{3}H] acetylcholine from choline and [*methyl*-^{3}H] acetyl-CoA in the incubation medium. When pure carnitine acetyltransferase was used, the amount of [*methyl*-^{3}H] acetylcholine produced was negligible. It may be concluded, therefore that although the presence of carnitine acetyltransferase in tissue extracts may account for the high activity in choline [illegible] controls, it will not contribute to the measurement of acetylcholine synthesized.

Statistics

Student's *t*-test for paired comparisons was used. *P* values less than 0.05 were considered significant.

RESULTS

There were no differences in body weights between the rats with ligated salivary ducts and their control litter mates, either at the start or at the end of the experiment; during the experimental period all animals gained about % in weight. In contrast, the rats from which the auriculo-temporal nerve had been removed were found to have lost 2% in weight.

Gland weights

The weight of the right duct-ligated submaxillary glands was only 31% of their contralateral unligated glands and 34% of the right glands of control rats (Table 2). The left submaxillary glands in "duct-ligated" rats weighed 10% more than the left glands of control rats. In control rats there was no difference in weight between right and left submaxillary glands.

The pattern was similar for the parotid glands (Table) the right duct-ligated glands weighing 44% of their contralateral unligated glands and 49% of the right glands of control rats. The left parotid glands in "duct ligated" rats weighed 17% more than the left glands of control rats. In control rats there was no difference in weight between right and left parotid glands.

Parasympathetic denervation caused a decrease in weight of the parotid gland of 40% ($P<0.001$) the right denervated gland weighed 99 ± 10 mg ($n=5$) and the contralateral gland 164 ± 13 mg ($n=5$).

Choline acetyltransferase activity

Enzyme activity in the right duct-ligated submaxillary glands was found to be 73% of that in the contralateral glands and 78% of that in the right glands of control rats (Table 3). The enzyme activity of the left submaxillary glands in the "duct ligated" rats did not differ from that of the left glands of control rats. In the control rats there was no difference in enzyme activity between left and right submaxillary glands.

In the duct-ligated parotid glands the enzyme activity was 81% of that in the contralateral glands (Table 3). When compared with the enzyme activity in the right glands of control rats the activity of the duct-ligated glands tended to be lower ($0.05<P<0.1$) but were not significantly so. There was no significant difference in enzyme activity between the left parotid gland in the "duct ligated" rats and that in the left gland in control rats; neither was there any difference in enzyme activity between left and right parotid glands of control rats.

In the parasympathetically denervated parotid glands the choline acetyltransferase activity was about 1% of that in the contralateral glands. In the denervated gland the activity was 0.6 ± 0.5 nmol acetylcholine synthesized h ($n=5$) and in the contralateral gland 57.0 ± 6.5 nmol acetylcholine synthesized h ($n=5$).

[illegible] ligated/ [illegible] control, %	Left unligated/ Left control, %
[illegible]	109.6±3.9 $P<0.05$
[illegible]	117.4±5.0 $P<0.01$

3 [illegible]

DISCUSSION

In order to measure the activity of choline acetyltransferase in salivary glands it was necessary to include control incubations in which the acetyl-

Table 1 *Incubation medium for choline acetyl transferase assay*

Substance added	Final concentration (mM) in incubation mixture Test	Control
Na-Phosphate buffer pH 7.8	33 0	33 0
NaCl	300 0	300 0
EDTA	0.5	0.5
Choline chloride	12.5	1?.5
Bovine plasma albumin	(1 mg/ml)	(1 mg/ml)
Dithiothreitol	2.0	2.0
Acetyl-CoA	0 5	0.5
Eserine sulphate	0 13	–
Acetylcholinesterase	–	(0.5 units)*
Final volume	(120 µl)	(1?0 µl)

One unit is the enzyme activity that hydrolyses 1 µmol acetylcholine per minute

Preparation of the gland

The glands were homogenized in 1 ml Na-phosphate buffer 0 1 M pH 6.5 containing dithiothreitol 1.2 mM using a Polytron homogenizer at high speed for 15 s The homogenate was either analysed immediately or stored at −20°C for periods up to 1 month without loss of activity In some cases the homogenates were centrifuged at low speed (900 g) for 10 min in order to remove cell debris. This procedure did not alter the activity of choline acetyl transferase but was not used routinely Ether treatment (Hebb Mann & Mead 1975) of the whole homogenate was tested but did not increase the enzyme activity

Estimation of choline acetyltransferase (acetyl-CoA choline *O*-acetyltransferase EC 2.3 1 6)

[*Methyl* H] Acetyl-CoA was prepared from CoA by treatment with [³H] acetic anhydride (see Hebb et al 1975) The specific activity measured in a refrigerated Packard scintillation spectrometer model 33?0 (counting efficiency ca. 50%) was 10 mCi/mmole

The incubation medium (Table 1) was that of Hebb et al (1975) with the routine addition of dithiothreitol (Calbiochem Ltd London) to a final concentration of mM

Assays were carried out in duplicate each tube contained 85 µl of incubation medium and 25 µl of tissue homogenate The reaction was started by addition of 10 [*methyl* H] acetyl-CoA (0.5 mM final concentration) a after 20 min at 39°C it was stopped by addition of 80 HClO 0 15 M followed by cooling in an ice bath.

[*Methyl* H] acetylcholine formed was separated fr [*methyl* H] acetyl-CoA on columns (0.65×10 cm) DeAcidite FFIP resin in the chloride form.

When controls containing incubation medium witho tissue homogenate (replaced by water) were used t enzyme activity of glands with non-ligated ducts w found to be at least twice that obtained in previous studi using a bioassay method (see e.g. Ekström 1973 197 Furthermore with this type of control an unexpected small reduction (about 60%) in enzyme activity was four in the parasympathetically denervated parotid gland (s Ekström 1978) These findings raised the questic whether the method was measuring only acetylcholi formation When additional controls without choline we tested with tissue homogenate from normally innervate glands it was found that about 50% of the radioactiv product was still produced and with homogenates of d nervated parotid glands it approached 100% To exclu the possibility of measuring the synthesis of radioactiv products other than acetylcholine a more specific contr was indicated and therefore acetylcholinesterase (0 5 unit per tube of type V S supplied by Sigma London Chemic Co. Ltd Poole Dorset) was added to each control whi eserine was omitted The choline acetyltransferase ac tivities given in the results section were obtained by sub tracting the control value from that of the test Simila controls have been used by Welsch (1977) in studies o placental choline acetyltransferase The compositions c the test and control incubation media is given in Table 1

The enzyme activity is expressed in nmol acetylcholine produced/gland h (total activity) the figures being corrected for the tissue volume

Carnitine acetyltransferase (acetyl-CoA Carnitine *O* Acetyltransferase EC 2.3 1 7)

The activity of carnitine acetyltransferase in tissue homogenates was measured by substituting carnitine (obtained from the Boehringer Corporation (London) Ltd Lewes Sussex) 1 mM final concentration for choline in the incubation medium The production of [*methyl* ³H acetylcarnitine was measured in the same way as [*methyl*

Table 2 *Effects of duct ligation on the weight (in mg) of salivary glands of rats*

Values are expressed as mean ± S E Number of observations is indicated in brackets

	Duct-ligated rats			Control rats		
	Right ligated	Left unligated	Right ligated/ Left unligated %	Right control	Left control	Right control/ Left control %
Submaxillary	68.8±3.2 (10)	226.5±8 6 (10)	31 0±2.3 $P<0\ 001$	?06. ±5 6 (10)	207.–±5 6 (10)	99 6±1.5 $P>0\ 1$
Parotid	74 0±3 0 (10)	172.1±8.6 (10)	43 9±2 6 $P<0\ 001$	151 6±5 1 (10)	146.5±3 6 (10)	103 7±3 3 $P>0\ 1$

[illegible]acetylcholine. These conditions were not necessarily [illegible] for carnitine acetyltransferase but gave good val[illegible] for pure enzyme (obtained from the Boehringer Cor[illegible], London).

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[illegible]

[illegible] test for paired comparisons was used. *P* values [illegible] 0.05 were considered significant.

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DISCUSSION

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Table 3 *Effects of duct ligation on choline acetyltransferase activity (nmol acetylcholine/gland h⁻¹) salivary glands of rats*

Values are expressed as mean ± S E Number of observations is indicated in brackets

	Duct ligated rats			Control rats		
	Right ligated	Left unligated	Right ligated/ Left unligated %	Right control	Left control	Right control/ Left control, %
Submaxillary	40 1±1.8 (9)	59 9±5 4 (9)	72.7±7 8 $P<0\ 01$	55 2±4 4 (9)	55 8±3.3 (9)	100 7±8.7 $P>0.1$
Parotid	44 3±3.2 (9)	55 7±2 2 (9)	80 6±6.9 $P<0\ 02$	53 6±2 8 (9)	50.9±4 0 (9)	110.5±9 7 $P\sim0\ 1$

choline formed by the tissue homogenates was continuously destroyed by the action of acetylcholinesterase The amount of radioactivity left in the controls was then subtracted from that obtained in the test incubations where acetylcholine was preserved The tissue homogenates were found to have considerable carnitine acetyltransferase activity this suggests that in the presence of endogenous carnitine acetylcarnitine may contaminate the radiochemical assay of choline acetyltransferase in the salivary glands Such a possibility has also been considered by the radiochemical assay of choline acetyltransferase in the heart (White & Wu 1973) and placenta (Welsch 1977)

Using the control incubations described above the choline acetyltransferase activity was found to be very low in denervated parotid glands a finding which agrees with previous observations (see Ekström 1978) The remaining enzyme activity about 1% of that of the contralateral gland can be accounted for since some nerves persist in the gland after the denervation procedure (Alm & Ekström 1976)

In the present study it was necessary not only to consider the effect of duct ligation on the treated gland but also the effect of this ligation on the contralateral control gland In a previous study (Ekström 1975) where the ducts of several major salivary glands were tied in an animal the intact gland(s) gained in weight and showed increased choline acetyltransferase activity compared with control glands in unoperated animals this was thought to reflect increased traffic of secretory impulses in the nervous pathways to the gland (see Ekström 1978) In this study the contralateral glands were also found to weigh slightly more than the corresponding glands of control rats but the increase in secretory drive was apparently not large enough to cause the choline acetyltransferase activity to increase significantly in these contralateral glands

In the duct-ligated submaxillary gland the choline acetyltransferase activity was reduced in comparison with both the activity in the contralateral gland and that of the corresponding gland of the control rat This is in contrast to the results of Ohlin & Perec (1967) who did not find the enzyme activity to decrease in the submaxillary glands of rats after duct ligation It should be noted however that these authors examined the glands only one week after ligation whereas in the present study the glands were examined 3 weeks postoperatively The effect of duct ligation on the weight and choline acetyltransferase activity of the parotid gland was not as marked as on the submaxillary gland in comparison with that in the corresponding parotid glands from control rats the enzyme activity tended to be lower but the difference was not statistically significant

Duct ligation causes a considerable derangement of the salivary parenchyma including cell atrophy and death (Tamarin 1967 Donath et al 1973) Recently Garrett Moghadam & Donath (1978) reported that the secretory cells of the parotid glands in rats loose their intimate relation with the nerves after duct ligation however no gross degeneration of the nerves was evident The small decrease in the choline acetyltransferase activity found in the present study also supports the idea that there is no marked nerve degeneration Disappearance of some of the nerve terminals cannot however be excluded as a possible explanation for the fall in the enzyme activity Another possibility is that a reduced flow of nerve impulses to the duct-ligated

...ligated/ ...control, %	Left unligated/ Left control, %
±15	109.5±13.8
18	P>0.1
±11	117.3±15.1
11	P>0.1

...gland may have caused decreased enzyme activity (see Ekström 1978). Afferent nerve impulses have been shown to arise from salivary glands (Kawamura & Funakoshi 1964, see also Emmelin 1967) and it is possible that the high pressure in the gland resulting from the obstruction of salivary flow may activate receptors within the gland, causing central inhibition of reflex stimulation and thereby reducing secretory impulses to the gland.

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Blood flow in proximal femur of the dog determined by the local ^{133}Xe injection method

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LAHTINEN, T., ALHAVA, E. M., HAKUMÄKI, M. O. K., JÄÄSKELÄINEN, J., VÄÄNÄNEN, A. & KARJALAINEN, P. Blood flow in proximal femur of the dog determined by the local ^{133}Xe injection method. Acta Physiol Scand 1979. 106: 437–440. Received 2 Febr. 1979. ISSN 0001-6772. Departments of Clinical Physiology and Surgery, Kuopio University Central Hospital and Department of Physiology and Medical Physics, University of Kuopio, Finland.

A new approach for measuring blood flow in bone is presented. It consists of the local injection of ^{133}Xe into the proximal femur of adult beagles and the external measurement of isotope washout curves. The curves were analyzed using a two-compartment exponential model. The half-times for the tracer washout from the fast and slow compartment were 3.34±0.48 min and 33.6±11.7 min (mean ±1 S.D.) respectively. Blood flow was calculated using a measured blood to bone partition coefficient of 1.55 ml/g. The mean blood perfusion in proximal femur of the dog was 10.8±4.3 ml/100 g/min (mean ±1 S.D.). This value agrees well with most estimates of bone circulation. Due to rapid disappearance of ^{133}Xe the method is easy to repeat and is thus suitable for physiological studies of local blood flow in bones.

Key words: Bone blood flow, femur, ^{133}Xe, partition coefficient

Quantitative measurements of the blood supply of bone cover a wide range of flow values. Most of the results are from animal rather than human experiments because of the traumatic character of the methods used. None of these methods are in common use although the indicator fractionation technique (Brookes 1970, Tothill & McCormick 1976), and methods based on blood clearance and skeletal uptake of bone-seeking radionuclides (Wootton et al. 1963, Van Dyke et al. 1965, Fredrickson, Honour & Copp 1955) seem to be, at this moment, the most used.

With a few exceptions most methods are based on the use of radioactive substances. The most popular are the gamma-ray emitting tracers, because such tracers are easily detected outside the bone with external detectors.

There are many ways to get the tracer into the bone. These include intra-arterial injection of tracer such as radioactive microspheres into the systemic circulation or selective injection into an afferent vessel of bone, intravenous injection of bone-seeking substances and the local tracer injection technique. One advantage of the latter technique is that extraosseous activity does not affect the measurements. Common to all of the local injection methods is the determination of the washout rate of the tracer from the injection site, because the rapidity with which the activity diminishes is dependent upon the blood flow. The results obtained by these methods have in most cases been indices, not absolute values of bone blood flow.

Semb (1971) has measured the initial slope of the ^{133}Xe elimination curves in studying bone marrow blood flow in uppertibial metaphysis of dogs but did not consider the method reliable for predicting bone marrow circulation. In this work a local ^{133}Xe injection technique has been developed for dogs. To obtain absolute values of blood perfusion in bone the partition coefficients of the two compartments of the xenon washout curves were determined.

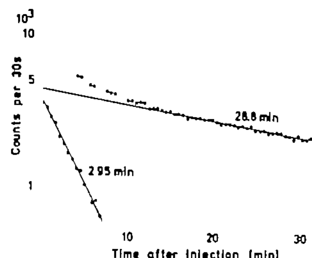


Fig 1 A typical ¹³³Xe washout curve from the proximal femur of dog.

MATERIAL AND METHODS

The experiments were made on 6 adult beagles (age 1 6±0 1 years, weight 11 1±1.2 kg (mean ±1 S D) The dogs were fasted overnight premedicated with morphine chloride (0.5 mg/kg) 0.5–1 h before the experiments and anesthetized with sodium pentobarbital (25 mg/kg)

The fur over the trochanteric region of the femur was removed and the bone exposed A hole about 1.5 cm deep was drilled with a Riordan fixation pin (diameter 1 2 mm) into the bone laterally A dose of 0 4 MBq of ¹³³Xe in saline solution (0 01–0 02 ml) was injected with a needle into the bone marrow and the hole was rapidly closed with a little piece of bone wax A collimated 2"×2" NaI(Tl)-crystal was placed in close proximity over the injection point of the bone The washout of xenon was followed for about 30 min with the detector coupled with a pulse height analyzer and a multichannel analyzer Nokia LP 4800 (channel width 30 s)

A measured washout curve of one dog has been plotted after background subtraction on a semilogarithmic scale and analyzed two-compartmentally (Fig. 1) The curves may be presented as the sum of two exponential terms

$$C(t) = \sum^{2} A_i e^{-\lambda_i t} \quad (1)$$

where A_i s are the initial values of the tracer activities present in the compartments at zero λ_i s are the decay constants of the exponential terms, respectively and are related to the observed half-times of the compartments by the expression

$$\lambda_i = \ln 2/T_{1/2,i} \; (i=1\;\; 2) \quad (2)$$

Blood flow of the measuring site can be calculated from the equation

$$F = \sum_{i}^{2} w_i \lambda_i k \quad (3)$$

where w_i s are the fractional flows of the compartments at zero ($w_1 = A_1/A_1 + A_2$ and $w_2 = A_2/A_1 + A_2$) and λ_i s the partition coefficients of the compartments

The partition coefficient measurements were perfor[med] by the method of Veall & Mallet (1965) The parti[tion] coefficient for the fast compartment has been estima[ted] to be the same as that of red cells For the slow comp[art]ment it was determined from the proximal femurs of se[...] beagles that were nearly of the same age as the dog[s] which the washout curves were measured

The bones were cleaned manually out of soft tissues [...] nearly the same sites where the curves had been measur[ed] a piece of bone approximately 5 cm was sawed [...] procedure for measurement of the partition coefficien[t of] bone has been described elsewhere (Lahtinen Ka[...] lainen & Väänänen) To calculate the coefficients, [...] solubility of xenon relative to air was determined for [...] blood cells plasma and saline The hematocrit of blo[od] inside the bone was chosen to be 40%

RESULTS

A typical washout curve of one dog is presented [in] Fig 1 The half times and the fractional flows of t[he] two compartments are shown in Table 1

The measured solubilities of xenon relative to [...] were 0 100±0 005 0 224±0 028 and 0 0896±0.0[...] (mean ±1 S D) for plasma red cells and salin[e] respectively A partition coefficient of 1.54 ml[...] was obtained for the first compartment assumi[ng] that it consists of red cells The measurements f[or] the second compartment bone gave a mean val[ue] of 1 56 ml/g when the density of bone was assume[d] to be 1 5 g/cm³ Because the values are close [to] each other a mean partition coefficient of 1.55 ml[...] has been used in calculations Using eq (3) t[he] blood perfusion of the trochanteric area was calc[u]lated to be 10 8±4 3 ml/100 g/min (mean ±1 S D[)]

In determining the partition coefficient of bone [a] partition coefficient of 14–15 ml/g for intraosseo[us] fat was obtained

Table 1 *Half times fractional flows and blood pe[r]fusions of bones of individual dogs and the corr[e]sponding means ±1 S D*

Dog no	$T_{1/2}$ (min) Fast comp	Slow comp		w	Flow (ml/100 g/min)
1	2 60	36.3	0 21	0 79	11 0
2	3 65	20 6	0 29	0 71	1 2
3	3 75	35 1	0 18	0 82	7 7
4	3 80	26 4	0 29	0 71	11 1
5	3.28	54 5	0 10	0.90	5 0
6	2 95	28 8	0 43	0.57	17 7
Mean	3.34	33 6	0 25	0 75	10 8
±1 S D	±0 48	±11 7	±0 11	±0 11	±4 3

DISCUSSION

The blood flow values presented in the literature cover a wide range of values the majority being in the range of 5–20 ml/100 g/min (Brookes 1970 [illegible] et al. 1963 Frederickson Honour & Copp 1955 Shim, Patterson & Copp 1971 Brookes 1967 White, Ter-Pogossian & Stein 1964 Kane 1968). Our results may thus be regarded as consistent with the values of former works. If the flow [illegible] ml/100 g/min, may be considered a mean perfusion value of the whole skeleton it may be roughly estimated according to Smith, Raymond & Wal-[illegible](1962) that about 6 % of the cardiac output flows to the skeleton.

The measured perfusion values in this work could not be compared precisely with flow values of earlier works. Firstly in many studies no distinction is made between bone and bone marrow circulations. The term, bone blood flow has not been accurately defined, and has been used although the measuring site, in addition to bone contains bone marrow either hematopoietic or fatty marrow Since the injection site in the presented method contains all of these tissues, we have used the term blood flow in proximal femur to describe blood circulation in the measuring site Secondly the comparison is difficult since only a few values of blood flow have been determined from the trochanteric region of femur In most cases the results are for whole femur and tibia or are presented as mean values for [illegible] bone. Thirdly the results are often those of animals other than dogs such as rats and rabbits. However the values are greatly influenced by the age of the animal (MacPherson & Tothill 1978)

Diffusible tracers, such as iodine-labelled antipyrine, xenon and krypton, have been widely used for estimation of blood flow These substances are rapidly dispersed throughout the organ and if there is tracer equilibrium between the tissue and venous blood, i.e. the blood to tissue partition coefficient is constant, the washout rate with which the activity is decreasing depends upon the blood flow In cases where the measuring field contains several compartments which are washed at different rates by the circulation, the recorded washout curves are multiexponential. Semb (1971) has noticed in his ¹³³Xe elimination curves of bone marrow several slopes but used only the initial one According to his measurements the mean half time of the initial slope was approximately 30 min which is near to our value of 33 6 min for the slow compartment However the results are not directly comparable because of different analyzing techniques The two-exponential model that we have applied assumes the measuring site to be composed of two compartments with different flows The washout from the first compartment was ten times faster than that of the second one The supposed compartments that might be physiologically meaningful are bone marrow especially the hematopoietic marrow and hard bone tissue including fatty marrow The partition coefficient of the first compartment, assumed to be associated with hematopoietic bone marrow was thus chosen to be the same as the partition coefficient of red cells. How the solubilities of xenon in red cells and hematopoietic marrow correspond to each other is not known The origin of the second compartment is supposed to be associated with hard bone tissue including fatty marrow The washout rate of the second compartment is slow because of the large partition coefficient of 14–15 ml/g of intraosseous fat.

We assumed that the surgery and drilling of the hole have little effect on blood flow and thus on the washout rates of the tracer in the measuring site In the same way it has been assumed that the bone wax stops the escape of the tracer into air and does not bind xenon which might markedly alter the washout rates. This was also verified by killing one dog used in other studies Practically no radioactivity vanished from the injection site during a 10 min follow-up

Since the measuring technique is a simple one and the disappearance of xenon out of bone is fast, the method could thus be applied for repeated physiological studies of regional blood flow in skeleton.

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Conversion of arachidonic acid to prostaglandins in homogenates of human skeletal muscle and kidney

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BERLIN T., CRONESTRAND R. NOWAK J SONNENFELD, T & WENNMALM Å. Conversion of arachidonic acid to prostaglandins in homogenates of human skeletal muscle and kidney Acta Physiol Scand 1979 106:441–445 Received 7 Febr 1979 ISSN-6772. Departments of Clinical Physiology and Surgery at Karolinska Institute and the Department of Urology Huddinge University Hospital Sweden.

The capacity of human skeletal muscle and kidney homogenates to synthetize prostaglandins (PGs) from exogenous precursor was investigated. Low-speed supernatants of muscle as well as renal medullary and cortical homogenates were incubated with ^{14}C-labelled arachidonic acid (^{14}C AA) prepared as sodium salt. ^{14}C PGs in the incubates were extracted, separated with thin-layer chromatography (TLC) and quantified by radioscanning. In the skeletal muscle incubates ^{14}C AA was converted into ^{14}C PGs with time-dependent yield, most effectively after 10–15 min incubation Well-defined radiopeaks parallel to unlabelled standards of PGD_2, PGE_2 $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were obtained in the chromatograms PGE_2 was the main PG formed, constituting over 50% of ^{14}C-activity whereas 6-keto-$PGF_{1\alpha}$ PGD_2 and $PGF_{2\alpha}$ were found in considerably lower proportions. In the renal medullary incubates, PGE_2 likewise accounted for the largest part of ^{14}C PGs formed, but significant relative amounts of $PGF_{2\alpha}$ and PGD_2 were also found. A minor peak, corresponding to 6-keto-$PGF_{1\alpha}$ and thus indicating formation of PGI_2 was also obtained. In contrast to the medulla, no ^{14}C PGs could be found in the renal cortical incubates. The results demonstrate the existence of considerable tissue specificity in the quantitative and qualitative expression of PG biosynthesis in man.

Key words Arachidonic acid kidney homogenate muscle homogenate prostaglandin formation

The existence of a considerable tissue specificity in the quantitative and qualitative synthesis of PGs in various animal tissues has been repeatedly reported in recent years (Pace Asciak and Rangaraj 1977 DeDeckere et al 1977 Blackwell et al. 1975). The finding is of great importance since different products of PG biosynthesis possess different biological properties and potency and hence their formation may reflect the physiological roles of endogenous PGs in different tissues. However besides these differences in the direction of PG synthesis the existence of considerable species differences also has to be taken into account. It is therefore of interest to verify the validity of previous animal experiments for human tissues and to define a specific profile of PG formation in these tissues In the present experiments the capacity of human skeletal muscle and kidney to form various PGs from exogenous AA has investigated

METHODS

The study was performed with the permission of the Ethical Committee at the Karolinska Institute. Muscle samples were obtained from 6 patients, 1 woman and 5 men, aged 50–73 years, operated on for obliterative arteriosclerotic disease with occlusion of either the common or the superficial femoral artery All the patients were fully informed of the nature, purpose and possible risks of the investigation before giving their voluntary consent to participate.

From 2 patients one 71 years of age with a malignant renal tumor and the other 21 years of age with hydronephrosis, kidney samples were obtained after nephrectomy

Preparation of tissue homogenates and incubation. Samples of muscle tissue (approximately 5 g) were taken peroperatively from non-ischemic areas of the middle portion of the quadriceps femoris muscle (m. vastus medialis) or from the lower portion of the rectus abdominis muscle. The samples were rapidly removed trimmed free of excess fat and connective tissue briefly washed and then immersed in 5 vol of ice-cold 0 1 M potassium phosphate buffer pH 7 4 They were subsequently minced and

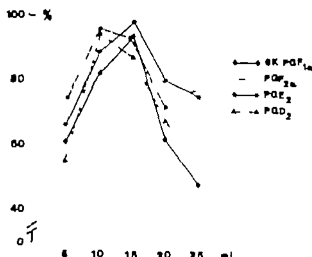


Fig 1 Yield of ^{14}C PGs during incubation of ^{14}C arachidonate with supernatants of skeletal muscle homogenate for different times The areas of the various ^{14}C PG radiopeaks in the chromatograms have been calculated separately in each incubation and are expressed as per cent of the maximal areas of the respective ^{14}C PG peaks obtained in the incubation series.

homogenized at 0°C in a Potter Elvehjem homogenizer with 5–6 up-and-down strokes of a teflon pestle motor driven at 300 rpm The resultant suspension was centrifuged at 4000×g for 30 min at +4°C to remove coarse cellular debris. The supernatant thus obtained was divided into portions corresponding to 1/4 of the muscle sample (about 6 ml) used for the incubation experiments Incubation was performed with 1.25 μCi Na-arachidonate freshly prepared from ^{14}C-arachidonic acid (New England Nuclear sp act. 40–60 mCi/mmol) at 37°C The incubation times were 5 10 15 20 or 25 min

Kidney samples were taken immediately after nephrectomy at the sites with macroscopically normal tissue morphology After removing connective tissue approximately 2 g of the renal medulla and cortex respectively were carefully isolated and immersed in 5 vol of ice-cold 0.1 M potassium phosphate buffer pH 7 4 The samples were then minced and homogenized as above The supernatants corresponding to 1/2 of the respective samples (about 5 ml) were incubated for 5 min with 1 25 μCi of Na-arachidonate The enzymatic reaction was terminated in all incubates by acidification to pH 3 5 with 0.5 M hydrochlorid acid The incubates were then submitted to the extraction and separation procedure

Extraction and separation After dilution of the incubates with 2 vol of water (to avoid foaming of the sample during extraction) prostaglandins were extracted twice with equal volumes of ethyl acetate (Fisher E 145) After evaporation of the organic phase the residue was reconstituted in 0.2 ml of ethanol and submitted to thin-layer chromatography (TLC) Chromatographic separation was performed using 0.25 mm DC Fertigplatten Kieselgel F 254 (Merck) in solvent ethyl acetate, acetic acid 2 2, 4-trimethylpentane water (90 20 50 100 v/v organic phase AIX Hamberg and Samuelsson 1966) against unlabelled standards of 6-keto-PGF PGF$_{1α}$ PGE$_2$, PGD and Na arachidonate Radioscans of the chromatograms were developed using a Berthold Dunnschicht Scanner The unlabelled PG standards were developed using 10 phosphomolybdic acid

Calculations

The areas of the various clearly defined radiopeaks th[at] corresponded to authentic PGs were calculated and e[x]pressed in per cent of the total area of ^{14}C PGs in th[e] respective radiochromatograms All values are given [as] mean±S E.

RESULTS

Extraction and purification

The efficiency of the lipid extraction procedure wa[s] controlled by determining the recovery of ^{3}H-labelled PGF$_{2α}$ and PGE$_2$ added to external standards The recovery of these compounds averaged 73±2% (n=3)

Thin layer chromatographic separation

Thin layer chromatographic separation of PGs was checked using unlabelled authentic compounds Complete separation between the PGs 6-keto-F$_{1α}$, D$_2$ E$_2$ and F$_{2α}$ was obtained with the solvent system used (A IX)

Effect of incubation time

The yield of the various ^{14}C prostaglandins formed during incubation of the supernatants of the muscle homogenates with ^{14}C-arachidonate varied with the

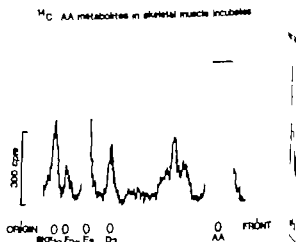


Fig 2 Radioscanning of a typical thin layer chromatogram of the products formed after 15 min of incubation of the supernatant of a muscle homogenate with ^{14}C arachidonate The letters 6-KF F$_{2α}$ E$_2$ and D$_2$ refer to the authentic prostaglandin standards

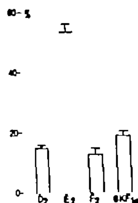


Fig. 3. Relative distribution of the ^{14}C PGs formed during 5 min of incubation of supernatants of muscle homogenates with ^{14}C-arachidonate. The bars indicate mean±S.E. of the radiopeak areas obtained in 5 expts. (TLC, system A IX) and are expressed as per cent of the total area of the ^{14}C PG peaks in the respective expts.

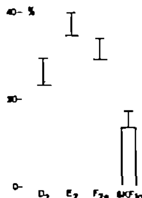


Fig. 5. Relative distribution of the ^{14}C PGs formed during 5 min of incubation of supernatants of the renal medullary homogenates with ^{14}C-arachidonate. The bars indicate mean±S.E. of the radiopeak areas obtained in 2 experiments (TLC, system A IX) and are expressed as per cent of the total area of the ^{14}C PG peaks in the respective experiments.

incubation time (Fig. 1). As seen from Fig. 1 radiochromatograms of 10 or 15 min incubates usually displayed the largest total ^{14}C PG area.

Products of incubation with ^{14}C-arachidonate

Incubation of the supernatants of the skeletal muscle and renal medullary homogenates with ^{14}C AA resulted in the appearance of various labelled compounds in the incubates. Radiochromatograms of the lipid extracts of these incubates revealed that the ^{14}C-activity usually appeared in 4 clearly defined peaks, corresponding to 6-keto-PGF$_{1\alpha}$, PGF$_{2\alpha}$, PGE$_2$ and PGD$_2$ (Figs. 2 and 4). In contrast, the chromatograms of the lipid extracts of the renal cortical homogenates, apart from a radiopeak corresponding to Na-arachidonate, displayed no clearly defined radiopeaks. In the radiochromatograms of the skeletal muscle incubates the largest radiopeak was usually found in parallel to PGE$_2$ (53±3%). The prominent PGE$_2$ peak contrasted to the considerably lower peaks corresponding to 6-keto-PGF$_{1\alpha}$ (19±1%), PGF$_{2\alpha}$ (13±2%) and PGD$_2$ (15± %, Fig. 3).

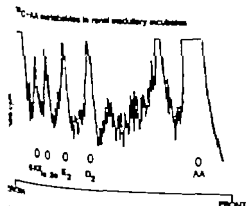


Fig. 4. Radioscanning of the thin layer chromatogram of the products formed after 5 min of incubation of the supernatant of renal medullary homogenate. The letters 6KF$_{1\alpha}$, F$_{2\alpha}$, E$_2$ and D$_2$ refer to the authentic prostaglandin standards.

The PGE$_2$ peak usually displayed the largest amplitude (34±5%) also in the renal medullary incubates. In addition, considerable relative amounts of PGF$_{2\alpha}$ (29±3%) and PGD$_2$ (23±6%) were found (Fig. 5). A minor peak was observed in parallel to 6-keto-PGF$_{1\alpha}$, indicating formation of PGI$_2$ (13±4%).

DISCUSSION

In the present experiments ^{14}C-labelled AA was incubated with low-speed supernatants of skeletal muscle, renal medullary and renal cortical homogenates. These homogenates certainly contained enzymes not only from tissue-specific cells but also from other non-specific tissue components that could not be separated from the samples such as blood vessels, connective tissue and nerves. This

implies that the PG bioformation in the incubates—apart from the enzymatic activity of specific cells—also to some extent reflects the metabolic capacity of all these non-specific components

The present chromatograms clearly demonstrate that the homogenates of human skeletal muscle and renal medulla possess the enzymatic capacity to synthetize PGs of the D_2 E_2 $F_{2\alpha}$ and I_2 (as reflected by the appearance of 6-keto-$F_{1\alpha}$) series from exogenous precursor The main PG formed by the homogenates of both tissues was PGE_2 and only minor amounts of 6-keto-$PGF_{1\alpha}$ were found The relative distribution of the ^{14}C PGs formed in the present experiments accordingly differs from data obtained in a previous study performed in our laboratory That study concerned the local conversion of intraarterially infused ^{14}C labelled AA to ^{14}C PGs in human forearm and kidney (Nowak and Wennmalm 1979) 6-keto-$PGF_{1\alpha}$ was the major product of the AA metabolism in both forearm and renal vasculature whereas only minor proportions of PGE_2 were detected A possible explanation for this discrepancy can be pointed out In experiments where ^{14}C AA is infused intraarterially vascular tissue is probably exposed to a considerably larger proportion of ^{14}C AA compared to experiments where the tissue is homogenized and incubated with ^{14}C AA This assumption is based on the fact that in infusion experiments vascular tissue is exposed to all ^{14}C AA infused while in homogenization experiments such tissue is exposed to ^{14}C AA only in relation to its relative weight in the tissue Therefore in the above mentioned infusion expts, vascular formation of ^{14}C PG probably accounted for a larger part of the ^{14}C AA conversion than in the current incubations of tissue homogenate The main PG formed in vascular tissue is PGI_2 (Moncada et al 1977) The currently observed larger proportion of ^{14}C-6-keto-$PGF_{1\alpha}$ in the infusion compared to the homogenization expts is in accordance with that observation and consequently indicates that the formation of 6-keto-PGF observed in the infusion expts mainly reflects formation of PGI_2 in the vascular components of the tissue

The capacity of renal medulla to synthetize PGs of the E_2 $F_{2\alpha}$ and D_2 series has been demonstrated in several animal preparations (Hamberg 1969 Crowshaw 1971 Dunn 1976 Tal et al 1976) The present chromatograms provide evidence that a similar PG synthesis occurs in the human medulla and that PGE_2 PGF_2 and PGD_2 are the major human renal PGs One point however deserves attention The occurrence of 6-keto-$PGF_{1\alpha}$ in the present chromatograms indicates a significant formation of PGI_2 in the renal medulla. Recently it was shown that PGI_2 is generated by microsomes from human renal cortex (Whorton et al 1977 Remuzzi et al 1978) but the production of prostacyclin by human renal medulla has not been reported hitherto

In the current incubates of cortical homogenate with ^{14}C AA no formation of ^{14}C PGs was obtained. Although the failure to recover appreciable quantities of PGs from renal cortical incubates does not necessarily indicate the absence of any PG synthesis in the cortex there is at present no obvious explanation for the lack of synthetic activity The presence of PG synthetizing enzymes has been demonstrated in rabbit and human cortical microsomes (Larson and Änggård 1973 Remuzzi et al 1978) On the other hand the synthetic activity of the rabbit cortex has been shown to be much lower than that of medulla (Larson and Änggård 1973) If the synthesis rate is slow also in the human renal cortex it may have accounted for the failure to detect some ^{14}C PGs in the current renal cortical incubates

In summary the present data show that human skeletal muscle and renal medulla possess the enzymatic capacity to convert exogenous precursor to prostaglandins of the D_2 E_2 $F_{2\alpha}$ and I_2 series The distribution of the PGs formed differs between the two tissues indicating the existence of tissue specific differentiation of PG synthesis in man

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Hemodynamic changes during the development of sodium induced hypertension in subtotally nephrectomized rats

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Hemodynamic changes during the development of sodium-induced hypertension were investigated in male Sprague-Dawley rats after about 70% of the renal mass was removed. Throughout the four experimental weeks, subtotally nephrectomized rats on high sodium diet (750 mEq/kg) showed continuous rise in blood pressure up to the mean value of 178±9 mmHg In sham-operated animals on the high sodium supply the blood pressure did not increase as compared to sham-operated controls on the standard sodium diet (150 mEq/kg) In the hypertensive group the primary changes were urea retention and concomitant increase of serum osmolarity but the serum sodium concentration remained at the normal level These changes were followed by sustained enlargement of extracellular fluid and relative intravascular volumes, together with a simultaneous increase of heart rate and blood pressure. During high sodium intake the plasma renin activity in subtotally nephrectomized rats was suppressed to one fifth of that in sham-operated animals, but the renin substrate activity did not increase markedly

Key words Subtotal nephrectomy renal hypertension, serum osmolarity extracellular fluid volume intravascular volume renin-angiotensin system

After reduction of renal mass, the development of hypertension is markedly accelerated by a sodium load (Coleman & Guyton 1969 Douglas et al 1964 Gross 1971 Kanda et al 1971 Koletsky & Goodsitt 1960, Langston et al 1963). In subtotally nephrectomized rats, high sodium diet (750 mEq/kg) induced a continuous rise of blood pressure during the course of 4 postoperative weeks whereas a standard sodium diet (150 mEq/kg) increased the blood pressure only slightly In sham-operated rats, the high sodium supply did not elevate the blood pressure during an experimental period of the same duration (Ylitalo et al 1976) Similar findings are also reported in dogs with the exception that in subtotally nephrectomized dogs, which received 0 9% saline as a drinking fluid, the steady level of high blood pressure was already achieved within a few days (Coleman & Guyton 1969 Douglas et al 1964 Langston et al 1963) In partially nephrectomized rats the elevation of blood pressure has [illegible] found to be associated with an increase in blood volume and a decrease in hematocrit values (Danda et al. 1972 Giffith & Ingle 1940 Ylitalo et al. 1976) In subtotally nephrectomized dogs on 0 9% saline an expansion of blood and extracellular fluid volumes has also been reported, but it is transient and lasts for only about 2 weeks after the operation (Douglas et al 1964 Guyton et al 1969) During high sodium intake the variation in some other parameters is poorly documented in subtotally nephrectomized rats and the findings obtained from dogs are not directly applicable to rats since the changes in parameters tested seem to be somewhat different in these species. Furthermore there are only very few experimental data in any species about the relation of blood pressure to many other parameters which

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are changed simultaneously with the development of hypertension induced by a prolonged sodium load Neither can findings in subtotally nephrectomized animals on a standard sodium supply be well applied to the problem of renal hypertension since their blood pressure is increased only slightly or at most moderately and thus relates poorly to possible changes in other hemodynamic variables (Douglas et al 1964 Guyton et al 1969 Langston et al 1963 Ylitalo et al 1976)

In the present study the development of hypertension and the correlations of blood pressure to the severity of renal insufficiency and to several hemodynamic parameters were followed in subtotally nephrectomized rats on high sodium diet during 4 weeks Changes in these parameters were compared with corresponding variables in sham-operated rats on either the standard or high sodium diet

MATERIAL AND METHODS

Male Sprague Dawley rats (SIV 50 strain) weighing 140–180 g were used The animals were kept in individual cages and given food containing 150 mEq sodium/kg (standard sodium diet) or 750 mEq sodium/kg (high sodium diet) (Möhring & Möhring 1977) The potassium content was 150 mEq/kg in each diet Demineralized water was available ad libitum

Subtotal nephrectomy was performed under ether anesthesia as described previously (Dauda et al 1972) the right kidney was removed and subsequently the upper and lower poles of the left kidney were cut off by a scalpel blade leaving about 30% of the total renal mass Bleeding was stopped by thermocoagulation The approximate weight of the remaining renal tissue was calculated on the basis of the removed tissue assuming that the right and the left kidney had equal weights. Control rats were sham-operated by exposing both kidneys

The animals were divided into 3 groups (1) sham-operated controls on the standard sodium diet ($n = 40$) (2) sham-operated rats on the high sodium diet ($n = 40$) (3) subtotally nephrectomized rats on the high sodium diet ($n = 43$) Each group consisted of animals killed on the 3rd 7th or 28th day after subtotal nephrectomy or sham operation.

Water consumption and body weight were measured daily between 8 and 10 a.m Systolic blood pressure and heart rate were recorded from the tail of unanesthetized rats twice during the first experimental week and then at intervals of one week by means of a piezoelectric crystal (W+W Electronic Basle) (Gerold & Tschirky 1968) after prewarming the animals at 29°C for 10 min

Extracellular fluid volume was determined in part of the rats killed on the 3rd 7th or 28th day after the subtotal nephrectomy and plasma volume in the remaining part of the animals killed on the 7th or 28th postoperative day

Extracellular fluid volume was measured by means of 10% inulin solution (physiological saline as solvent) which was injected into the saphenous vein under light ether anesthesia (about 0 1 ml/100 g b.w.t.). The exact amount of solution administered was estimated by weighing the injection syringe before and after the injection. The excretion of inulin was prevented by isolating the kidney or kidney remnant carefully from systemic circulation with thread ligatures just before the inulin injection. After an equilibrium time of one hour the blood samples were taken from the cannulated carotid artery of ether-anesthetized rats and then centrifuged The inulin concentration of the supernatant was estimated according to the method of Schreiner (1950)

The plasma volume was determined by 0.5% Evans blue solution (physiologic saline as solvent) administered and dosed as inulin solution Ten minutes after the injection, blood samples were drawn from the carotid artery and centrifuged The dye concentration of the supernatant was measured photometrically (Eppendorf 1101 M Hamburg) Blood volume and packed blood cell volume were calculated on the basis of plasma volume and hematocrit according to the formula of Wang (1959).

In each animal the following parameters were measured from the blood samples drawn from carotid artery hematocrit in heparinized capillary tubes by centrifugation sodium concentration of serum by flame photometry (FAII flame photometer and Zeiss M4QII spectrometer Jena) in the dilution 1 ?0, urea concentration of serum according to the method of Fawcett & Scott (1960), using commercially available kits (Boehringer Mannheim Ltd Mannheim) and serum osmolarity by determining the freezing point of the serum samples (Knauer Osmometer type M Berlin)

The activities of plasma renin and renin substrate, in blood samples collected from carotid artery of ether anesthetized rats were measured in part of the animals killed on the 28th experimental day ($n=8$) Renin and renin substrate activities were determined by means of radioimmunoassay of angiotensin I released in incubation medium within 1 h Plasma samples for renin determination were incubated according to the micromethod of Boucher et al (1967) For the incubation of renin substrate the method of Carratero & Gross (1967) was used

The results are presented as means ± standard error (S E) Statistical evaluation was performed using the Student s t-test or t-test for paired observations Regression equations were calculated by the least squares method

RESULTS

Subtotally nephrectomized rats on the high sodium diet showed a continuous rise in their blood pressure up to the mean value of 178±9 mmHg whereas in sham-operated animals the blood pressure was not elevated at all (Fig 1A) During the first experimental week, the heart rate varied but not significantly Thereafter the heart frequence in operated rats increased but in both sham-operated

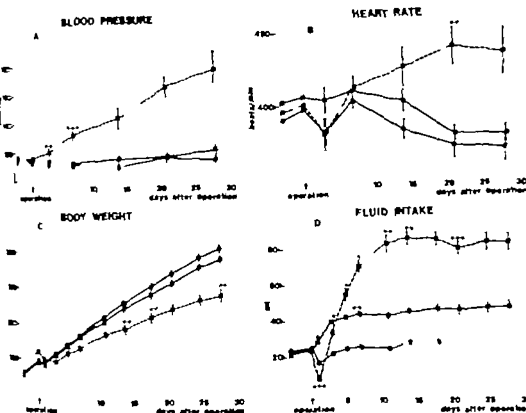


Fig. 1. Blood pressure (A), heart rate (B), body weight (C), and fluid intake (D) in the groups studied (0–3 days n=40–43, 4–7 days n=32–34, 8–28 days n=17–18). Subtotally nephrectomized rats on high sodium diet (■- -■) are compared with sham-operated animals on high sodium diet (■—■) and the latter group with controls on standard sodium diet (●—●). *P<0.05 **P<0.01 P<0.001

[illegible] it showed a decreasing tendency (Fig. 1B). In the operated group there was a significant correlation between blood pressure and heart rate at the end of the experiment (Table 1).

In subtotally nephrectomized *rats* the daily *body weight gain* was smaller than in both sham-operated groups, and at the end of the expt. the body weight correlated inversely to the height of blood pressure (Table 1). During the last experimental week, the sham-operated animals on the high sodium diet had a somewhat lower body weight than controls on the standard sodium diet (Fig. 1C).

After an initial decrease following the operation, the *fluid intake* of subtotally nephrectomized rats was almost doubled, compared to that of sham-operated animals on the high sodium diet, the latter group showing water consumption about 60% higher than controls on the standard sodium diet (Fig. 1D). Three days after the subtotal nephrectomy the fluid intake correlated inversely to serum urea concentration and osmolarity whereas later on no such correlation could be found. At the end of the expt. the operated animals with the lowest renal function unexpectedly had the lowest water consumption, and a positive correlation between fluid intake and body weight was found (Table 1).

After the first experimental week, *the hematocrit* of the subtotally nephrectomized rats was lower than that of both sham-operated groups, and during the course of the expt., this difference became more pronounced. No difference in hematocrit values was seen between the sham-operated groups (Fig. 2A). In the operated group a negative correlation between hematocrit and blood pressure or heart rate was found at the end of the expt. (Table 1).

Although some variation in *serum sodium concentration* was seen throughout the expt. there was no significant difference between the various groups (Fig. B). Serum sodium concentration correlated significantly to no parameters studied.

Table 1 *Correlation coefficients between various parameters in subtotally nephrectomi ed rats on sodium diet*

		On the 3rd day			On the 7th day			
						Packed blood cell volume		O… p… m… st…
Parameters	*n*	Fluid intake 9	Serum osmolarity 9	Other parameters studied 8–43	Serum osmolarity 17	Absolute 8	Relative 8	8-
Blood pressure	9–17	n s	n s.	n s	n s	n s.	n s.	[illegible]
Heart rate	9–17	n s	n s	n s	n s	n.s.	n s	[illegible]
Body weight	9–17	n s	n s	n s	n s	n.s.	n s	[illegible]
Fluid intake	9–17		−0 815	n s.	n.s	n.s	n s.	[illegible]
Hematocrit	9–17	n.s	n.s	n.s	n.s	n.s	0 793	[illegible]
Serum urea	9–17	−0 857*	0 850*	n.s	0 748*	n.s	n s.	[illegible]
Serum osmolarity	9–17	−0.815		n.s		n.s	n s.	[illegible]
Extracellular fluid volume								
Absolute	8	n s	n.s	n s.	(0 678)			[illegible]
Relative	8	n s	n s.	n.s	n s.			[illegible]
Plasma volume								
Absolute	8	–	–	–	n s.	n s	n.s	n
Relative	8	–	–	–	n s.	n s	n s	n
Packed blood cell volume								
Absolute	8	–	–	–	n s.		0 828*	[illegible]
Relative	8	–	–	–	n.s.	0 828*		[illegible]
Intravascular volume								
Absolute	8	–	–	–	n s.	0.749*	n s	n.
Relative	8	–	–	–	n.s	0 798	(0.645)	n.

– = Either of parameters not measured in same rats n s. = not significant Coefficient in parentheses. $P<0.1$ *P* $P<0\ 01$ $P<0\ 001$

Throughout the expt *serum urea concentration* in the subtotally nephrectomized rats was about 3 times higher than in sham-operated animals Four weeks after the sham-operation the urea level in rats on the high sodium diet was slightly higher than that of controls on the standard sodium diet (Fig 2C) In the operated rats *serum osmolarity* increased concomitantly with the serum urea concentration and during the course of the expt a very significant correlation between serum osmolarity and urea level was found No difference in serum osmolarity was found between the two sham-operated groups (Fig. 2D) Four weeks after the subtotal nephrectomy both serum urea and osmolarity correlated directly to blood pressure and inversely to hematocrit (Table 1)

In the subtotally nephrectomized rats some expansion in relative *extracellular space* (ml/kg b wt) was seen on the 7th postoperative day At the end of the expt absolute and relative extracellular fluid volumes had increased 19% and 43% respectively compared to the sham-operated rats on the high sodium diet No differences in extracellular fluid volumes were found between the sham-operate groups (Fig 3 A) Four weeks after the subtou nephrectomy both absolute and relative extrace lular fluid volumes correlated directly to bloo pressure and inversely to hematocrit A positiv correlation between extracellular fluid volume an serum urea or osmolarity could also be calculate (Table 1)

Since the extracellular fluid volume in the subto tally nephrectomized rats was unaltered on the 3rd postoperative day it was not thought reasonable t measure *plasma and intravascular volumes* on th 3rd experimental day Seven days after the opera tion plasma and blood volumes were still un changed Three weeks later the relative plasma anc intravascular volumes (ml/kg b wt) in subtotally nephrectomized rats were 25% and 18% higher re spectively than the corresponding volumes in sham-operated animals on the high sodium diet anc showed a tendency to increase during the course of the expt at the 28th postoperative day they were markedly higher than on the 7th experimental day ($P<0\ 025$) The absolute plasma and intravascular

30th day

[illegible]	Heart rate	Body weight	Fluid intake	Hemato-crit	Serum urea	Serum osmolarity	Other parameters studied
	17	17	17	17	17	17	8–17
[illegible]							n.s.
[illegible]							n.s.
	s.	0.624**					s.
[illegible]	−0.525*	(0.422)	s				n.s.
[illegible]	0.493*	0.489*	s.	−0.767*			n.s
[illegible]			n.s	−0.695 **	0.859**		n
[illegible]		n.s.	n.s.	−0.758*	0.791	0.835	s.
[illegible]		s.	s	−0.707*	0.839**	0.816	s.
[illegible]	s.	n.s.	(0.705)	(−0.705)	(0.632)	(0.622)	s.
[illegible]		s.	n.s.	−0.884	(0.631)	(0.648)	n.s.
	n.s.	(0.590)	n.s.	n.s.	n.s.		n.s.
		s.	n.s.	n.s.	(−0.596)	−0.697*	n.s.
[illegible]		(0.672)	(0.690)	n.s.	n.s.	n.s.	n.s.
			n.s	−0.712*	n.s.	n.s.	n.s.

[illegible] volumes in the operated rats did not differ from those in the sham-operated groups. Neither was there any difference in the absolute and relative volumes between the sham-operated groups (Fig. [illegible]). Four weeks after the subtotal nephrectomy relative plasma and intravascular volumes correlated directly to blood pressure and inversely to hematocrit (Table 1). In subtotally nephrectomized rats on the high sodium diet the relative *packed blood cell volume* (ml/kg b. wt.) tended to decrease, but it did not differ significantly from that in the sham-operated groups. At the end of the expt. unexpectedly low cell volumes were however found in a few of the operated animals with the very low renal function.

During the high sodium intake the *plasma renin activity* of subtotally nephrectomized rat was depressed to 20% of that in sham-operated animals on the high sodium diet. The plasma renin activity in the latter group was not significantly lower than in controls on the standard sodium diet (Fig. 4). In subtotally nephrectomized rats activity (*plasma renin subst*[illegible]) [illegible] to increase but it differed significantly only from that in controls on the standard sodium diet ($P<0.05$). In subtotally nephrectomized rats plasma renin and renin substrate activity correlated significantly to none of the parameters studied.

DISCUSSION

Under the sodium load, the subtotal nephrectomy induced a continuous rise in blood pressure during the course of the expt. In sham-operated animals the high sodium intake did not increase blood pressure or heart rate. Four weeks after the subtotal nephrectomy blood pressure correlated directly to serum urea concentration and, consequently to the degree of renal insufficiency. The heart rate in the operated group showed a concomitant increase with blood pressure until the end of the expt. I[illegible] subtotally nephrectomized dogs (Coleman & Guyton 1969) and in unilaterally nephrectomized rats with contralateral renal artery constriction (Ledingham & Cohen 1964) however only a transi-

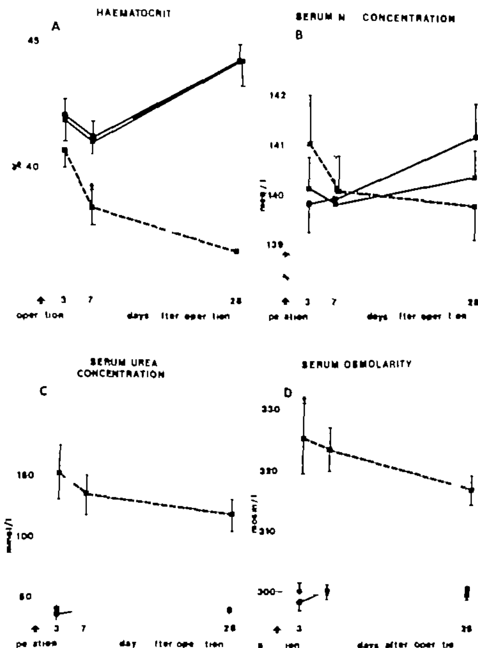


Fig 2 Hematocrit (A) serum sodium concentration (B) serum urea concentration (C) and serum osmolarity (D) in the groups studied (on the 3rd day $n=8$–9 on the 7th and 28th day $n=16$–17) Subtotally nephrectomized rats on high sodium diet (■- - -■) are compared with sham-operated animals on high sodium diet (■——■) and the latter group with controls on standard sodium diet (●——●) $P<0.05$ $P<0.01$ $P<0.001$

tory increase in heart rate during 2 postoperative weeks has been reported

At the end of the expt the body weight of operated rats correlated inversely to serum urea concentration and blood pressure Thus the high blood pressure together with the renal insufficiency retarded the body weight gain of rats

Three days after the subtotal nephrectomy the fluid intake was markedly decreased and correlate directly to renal function Thereafter daily wate consumption increased seemingly independently o the function of the kidney remnant In the operate rats with the lowest renal function however th unexceptedly low fluid intakes were measured These low fluid consumption values can be least partly explained by the finding that thos

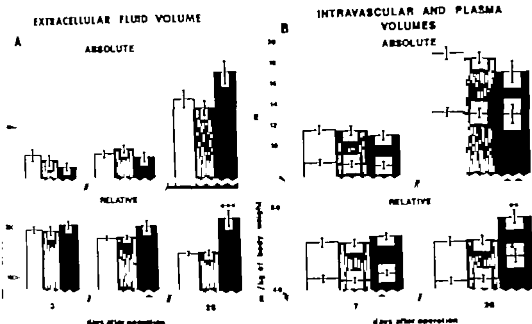


Fig. 3. Extracellular fluid volume (A), and intravascular and plasma volumes in the groups studied (in B the lower part of column describes plasma volume and the upper part packed blood cell volume; the total column describes intravascular volume). Subtotally nephrectomized rats on high sodium diet (■) are compared with sham-operated animals on high sodium diet (▥), and the latter group with controls on standard sodium diet (□); $P<0.05$ $^{**}P<0.01$ $P<0.001$

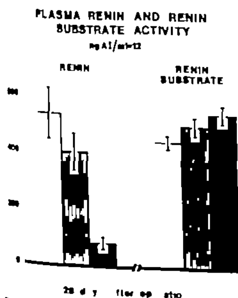


Fig. 4. Plasma renin and renin substrate activity in the groups studied at the end of the experiment (=8–9). Subtotally nephrectomized rats on high sodium diet (■) are compared with sham-operated animals on high sodium diet (▥), and the latter group with controls on standard sodium diet (□), $^{**}P<0.01$

animals also had the highest relative extracellular fluid volumes which might have antagonized the thirst stimulation (Fitzsimons 1977)

The primary change after the subtotal nephrectomy seemed to be the increase of serum urea concentration which, together with the probable accumulation of other products of metabolism was most likely responsible for the rapid elevation of serum osmolarity The high sodium diet induced no marked alteration in the serum sodium concentration, and thus cannot explain the increase in serum osmolarity This agrees with the concept of Langston & others (1963) that changes in serum sodium level per se are not apparently the primary cause of sodium-induced hypertension in subtotally nephrectomized dogs.

I operated rats the increase of extracellular fluid volume without any significant change in serum sodium concentration, indicates that water is retained concomitantly with sodium. At the end of the expt. the marked correlation between extracellular fluid volume and both serum urea concentration and osmolarity suggests that the expansion of extracellular pace was dependent on the degree of renal insufficiency and subsequently on serum

osmolarity Although the absolute plasma and in travascular volumes in operated rats were un changed the high relative volumes suggest water accumulation also in the intravascular space The low absolute volumes ensued from the quite low body weight of the operated animals in which the intravascular space was measured Our findings agree with the observations of Guyton and his as sociates obtained from subtotally nephrectomized dogs (Douglas et al 1964 Guyton et al 1969) with the exception that in our study the extracellular fluid and intravascular volumes remained at the in creased level until the end of the four week experi ment whereas in dogs the enlargement of these fluid compartments was found to last for only about 2 postoperative weeks The long-term expansion of extracellular and intravascular spaces in operated rats might be associated with the quite slow de- velopment of hypertension as compared to dogs in which the high blood pressure had developed more rapidly and achieved the steady level within some days This difference in the development of hypertension might be attributed to the fact that in our expts the sodium was given in the diet but in those dog expts in 0 9% saline as drinking fluid Thus the sodium load in our study was obviously milder than in conditions where sodium is given in isotonic salt solution instead of diet since the thirst stimulation caused by dietary salt and serum hyperosmolarity can be better compensated by an excess intake of demineralized water than by drink ing the isotonic saline The extracellular and in travascular spaces might however be readjusted later on to unexpanded volumes if the experiment were continued until the steady level of hyperten sion had been maintained for e g some weeks

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fluid compartments. The water retention and the sodium load suppressed the renin-angiotensin system in subtotally nephrectomized rats but did not significantly effect on the renin substrate activity in plasma. In this model of hypertension the primary change seemed to be the increase of serum urea concentration and the simultaneous elevation of serum osmolarity which probably resulted in the expansion of the extracellular and intravascular spaces, and consequently in the development of hypertension.

We are grateful to Miss Beate Korzenblaser and Mr H. Bil for their technical assistance.

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Influence of neural and humoral beta adrenoceptor stimulation on dynamic myogenic microvascular reactivity in cat skeletal muscle

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Analysis of myogenic microvascular reactivity in terms of its recently described prominent dynamic component was performed before and during graded sympathetic stimulation and catecholamine infusion. Phenoxybenzamine and propranolol were used to differentiate between α- and β-adrenoceptor effects. The study first confirmed previous findings of β-adrenergic inhibitory component in the neural control of microvascular resistance which attenuated the α-adrenergic constriction. The results concerning the interaction between adrenergic and myogenic control mechanisms corroborated the conclusion that the sympathoadrenal system, via its β-adrenergic link exerts effective inhibitory action on myogenic excitatory reactions As regards the neural control, its β-adrenergic component seemed to quite precisely compensate for the reinforcing effect on the myogenic constrictor response which results from increased vascular tone per se (in this case caused by α-adrenergic constriction) interpreted as physical gain effect inherent in the inverse fourth power relationship between radius and resistance. The latter complicating factor which implies non-linearity in integrated peripheral resistance control was thus revealed only after β-blockade, but not on the vascular bed with intact adrenoceptors, where given transmural pressure stimulus evoked an almost equally large myogenic constrictor response irrespective of the prevailing level of vascular tone. The β-inhibitory action of blood-borne noradrenaline was similar to the neural one whereas that of adrenaline was more effective, causing decline of myogenic reactivity below control

Key words. Myogenic control vascular control dynamic response, rate-sensitivity β-adrenoceptor inhibition, adrenoceptor influence, Poiseuille's law microvascular tone

It is a common empirical observation that myogenic vascular reactivity can be severely depressed under experimental conditions involving trauma. The mechanism behind this interference with myogenic responses, which is apparent even during slight stress, has been largely unknown. A recent study (Grände & Mellander 1979) indicated that this phenomenon, at least partly could be attributed to a β-adrenergic inhibitory interference with myogenic reactivity in turn caused by stress-induced catecholamine release from the adrenals.

In the present study the interaction between adrenergic and myogenic regulatory mechanisms was analysed more systematically and an attempt was made to reveal whether the β-adrenergic vascular control system might serve the function to modify myogenic reactivity also under normal physiological conditions For this purpose myogenic reactivity to a standardized transmural pressure stimulus was studied in skeletal muscle under concomitant graded adrenergic influence on the vascular bed within the physiological range Observations before and after adrenergic blockade were thus made during infusions of catecholamines and also during activation of the sympathetic nerve fibers which recently were shown to exert a significant β-adrenergic inhibitory influence on the tone of the microvessels (Lundvall & Järhult 1976) Such β-noradrenergic influence was revealed to result in a clear attenuation of the simultaneous α-adrenergic constrictor response in the microvascular resistance vessels and a dilation of the precapillary

[illegible] small [illegible] on the other. There is little doubt that such communicants exist on the venous side. Below we have suggested some arguments for the view that also on the arterial side there is sufficient communicant [illegible] to make the SAP measurements reliable and useful.

Morphological observations (e.g. Wiedeman 1963, Hammersen 1968, Eriksson & Myrhage 1972) have demonstrated the existence of communicating vessels between different branches of the cognate artery in skeletal muscle at the arteriolar and more distal level, i.e. between vessels of about 20–25 µm (I.d.) and smaller. There are also similar, though much less abundant, connections between branches of different feeding arteries in the muscle. This arrangement would indicate the presence of such vascular connections in the gastrocnemius muscle between the cannulated small distal sural artery and its more proximal, still patent side branches and also to some extent between the cannulated vessel and the microvessels of the popliteal artery. Evidence for this opinion was obtained from our observations of a profuse pulsatile outflow of arterial, well oxygenated, blood from the SAP catheter upon disconnection from the pressure transducer. Such flow of oxygenated blood, though reduced, was still present after temporary occlusion of the sural artery at the site of its proximal origin from the popliteal artery but it ceased upon occlusion of the latter vessel. It seems likely therefore that the demonstrated communicant flow is sufficient to nourish the muscle region that normally is mainly supplied via the cannulated distal sural artery and hence, that its own microvessels have well preserved tone and reactivity. If this were not the case, the measured SAP would be expected to approach SVP since the resistance of its draining vessels would be very small due to pronounced metabolic and myogenic vasodilatation. However, SAP in the control period averaged 66±3 mmHg in the present experiments which is compatible with the view that it adequately reflected pressure in arterial microvessels with an i.d. of 20–25 µm to judge from the diameter and direct micropipette pressure measurements of Fronek & Zweifach (1975). Note that our estimate of this deduced value for the size of the arterial microvessels (20–25 µm) coincides with the largest size of those vessels which have arterial communicants. Strong circumstantial evidence for the opinion that measured SAP is representative of arterial microvascular pressure in the whole muscle region was obtained from the finding in the present and in a previous study (Grände et al. 1977) that the prominent dynamic microvascular constrictor and dilator responses to transmural pressure stimulus were always simultaneously reflected in total regional vascular resistance, the latter necessarily referring to the whole muscle region. This finding strongly supports the view that the dynamic resistance effects to be described in response to transmural pressure stimulus are caused by active constrictions in the microvessels and not merely the result of a shift in position along the vascular tree from which SAP and SVP were recorded, nor due to passive regional redistribution of vascular resistance. It thus seems fair to conclude that microvascular resistance responses can be quite reliably recorded with the technique used here.

Mention should be made that in some pilot experiments, in which the sural artery was cannulated close to its origin, SAP was often quite low, sometimes approaching SVP. Under these conditions we recorded poor microvascular reactivity. This indicates impaired tone in the sural artery microvessels, apparently due to the stopped flow in the side branches emanating from its proximal stem and hence insufficient blood supply to the region from which SAP was recorded. It is therefore suggested that the present more distal cannulating technique is to be preferred in order to obtain reproducible data and, hence, more useful information from SAP measurements.

RESULTS

Dynamic myogenic reactivity in the microvessels (<20–25 µm) of skeletal muscle was analysed throughout this study in terms of the rate-dependent constrictor response evoked by a standardized dynamic myogenic stimulus accomplished by an increase in vascular transmural pressure (P_T) of 40 mmHg applied at the constant rate (dP_T/dt) of 2.5 mmHg/s (see Methods). This standardized transmural pressure stimulus will be denoted 'the dynamic test stimulus' below.

Fig. 1 illustrates characteristic rate-dependent microvascular constrictor responses to this test stimulus. The response in panel A represents control dynamic myogenic reactivity as evoked under normal resting conditions on the sympathectomized vascular bed with normal basal tone and at normal arterial (120 mmHg) and venous (≈5 mmHg) pressures. It was thus obtained after a resting period of 1 h after the preparation was finished, i.e. vascular tone can be considered to have returned to normal after the surgical trauma (Grände & Mellander 1979). The dynamic test stimulus increased microvascular resistance in this case by about 12 PRU (mmHg ml^{-1} min^{-1} 100 g) above the preceding control level. Panel B shows an almost identical resistance response to the same stimulus after an additional resting period of 30 min demonstrating the good reproducibility of the control dynamic microvascular response. Panel C illustrates the corresponding response after effective combined α- and β-adrenergic blockade confirming our previous findings (Grände et al. 1977, cf. Johansson & Mellander 1975) that the response is not affected by the blocking drugs per se, nor mediated via adrenergic mechanisms, and, hence, can be considered to be of local myogenic origin. It was also confirmed (cf. Grände et al. 1977) in these experiments and in those to be reported below that the dynamic micro-

sphincters (Lundvall & Järhult 1976 Lundvall & Hillman 1978) Since under normal resting control conditions the recently described dynamic myogenic response to a given strong test transmural pressure stimulus is much larger than the static response (Grände et al 1977 1978) and is highly reproducible emphasis in the present study was placed on dynamic myogenic reactivity and its interaction with adrenergic mechanisms

Since activation of the α-adrenergic control system leads to marked increase in vascular tone the present analysis had to take into account the fact that the amplitude of a superimposed (in this case myogenic) constrictor response is directly influenced by the level of vascular tone prevailing prior to its release (i e Myers & Honig 1969)

METHODS

The study was performed on 18 cats b wt 3 0–5 0 kg, anesthetized with α-chloralose (50 mg/kg b wt) and urethane (100 mg/kg b wt) The cats were breathing spontaneously through a cannula, except for experiments where the regional somatomotor nerves were blocked when respiration was supported by a respirator Body temperature was kept at 38±0.5°C

Experimental preparation The observations were made on the vascular bed of the acutely denervated lower leg muscles of the right hindlimb This muscle region was prepared so that the popliteal artery and vein formed the sole vascular connections with the body Surgical procedures and the experimental set up were basically the same as described previously (see Grande Lundvall & Mellander 1977) and will be only briefly described After heparinization (1 000 IU/kg b wt) the muscle region was perfused (non pulsatile flow) with a pressure servo controlled roller pump inserted in a shunt circuit between the femoral and popliteal artery permitting mean arterial inflow pressure to be adjusted to desired levels Mean arterial blood flow (Q) to the region was continuously recorded in the shunt circuit with a reliable pressure gradient flowmeter (Grände & Borgström 1978a) Venous blood flow from the region was diverted via a catheter to the right jugular vein Mean arterial inflow pressure (AP) and venous outflow pressure (VP) were measured from T-tubes close to the cannulated popliteal artery and vein. Pressures from arterial microvessels ('small artery pressure SAP) and from venous microvessels ('small vein pressure SVP) were continuously monitored using a technique modified from that originally described by Haddy et al (1954) For this purpose the sural artery and vein on the posterior surface of the gastrocnemicus muscle were ligated at a site just distal to the adjacent fat pad and flexible nylon tubings (i d of tip ~0 3 mm) were inserted just below these ligatures. With this technique the pressure monitored from these small catheters is supposed to adequately reflect microvascular pressure in the muscle region an assumption which will be further commented upon below All pressures were recorded with Statham transducers a the parameters were registered on a Grass Polygraph.

The muscle preparation with intact vascular conne tions was placed in a hermetically closed temperatur controlled plethysmograph (38°C) filled with Tyrod solution The fluid in the plethysmograph was connect via a tubing with an external open reservoir mobile in t vertical direction to permit variation of the hydrostat fluid pressure in the plethysmograph to desired levels relation to the atmospheric pressure (Grände Järhult Mellander 1974) In the control situation i e. at atmo pheric plethysmographic pressure VP was adjusted to level (≈5 mmHg) which established an isovolumetric sta of the preparation By lowering the reservoir all parts the muscle vascular bed could be exposed to increase vascular transmural pressure (P_t) and thi could b applied at any desired rate dP_t/dt Selective regor sympathetic nerve stimulation was accomplished b electrical stimulation (8 V 4 ms) of the distal end of th cut sciatic nerve after effective somatomotor blockad caused by a continuous close arterial infusion of pancur nium bromide (Pavulon® 0 01 mg kg min).

The following vasoactive and blocking substances we used adrenaline bitartr noradrenaline bitartr isopr terenol sulphate phenoxybenzamine (10 mg kg tissu propranolol chloride (1 mg kg tissue) and atropin su phate (1 mg kg tissue) Effective α and β-blockad was ensured by tests with supramaximal doses of norad renaline (5 μg kg tissue) and isoproterenol (5 μg kg tissue) respectively

Data are presented as mean values ± S E

Recordings of vascular resistance With the use of dif ferential pressure transducers (National Semic LX 1601D) and electronic divider circuits as described by Grände & Borgström (1978a) the present technique per mitted continuous recordings of total and segmenta resistances in the muscle vascular bed according to the following definitions Total vascular resistance (AP VP)/Q proximal arterial resistance =(AP–SAP)/Q, microvascular resistance =(SAP–SVP)/Q and large vein resistance =(SVP VP)/Q Arguments have been presented elsewhere (Grände et al. 1977 1978) for the opinion that this approach permits reliable recordings of the three first mentioned resistances even during phasic change of vascular transmural pressure Large vein resistance which is very small compared to total resistance (Grände et al 1977) requires correction for the effects of possible net transcapillary fluid movements and capacitance responses and such data can be obtained from plethysmographic volume recordings. Emphasis in this study was placed on microvascular resistance since this section exhibits dynamic myogenic reactivity as shown in the above mentioned studies

Comment on the validity of the indirect microvascular pressure measurements The method used to determine microvascular resistance presupposes that the SAP and SVP measurements are representative for pressures in the arterial and venous microvessel in the studied muscle region This in turn requires the presence of communicants between normally perfused arterial microvessels and the cannulated small artery on the one hand and between normally perfused venous microvessel and the



Fig. 2 Effects of graded sympathetic nerve stimulation on microvascular resistance (abscissa) and on the superimposed dynamic myogenic constrictor response (ordinate) to standardized test transmural pressure stimulus on the calf vascular bed with intact adrenoceptors (lower curve) and after effective β-adrenergic blockade (upper curve).

The results (mean values ± S.E., n = 6) are illustrated in Fig. 2. The microvascular resistance prevailing in the steady-state at the different nerve excitation rates is plotted along the abscissa and the superimposed dynamic myogenic constrictor response along the ordinate. Sympathetic nerve stimulation by itself caused an increase in microvascular tone graded in relation to the excitation rate both in the intact and the β-blocked vascular bed. This constrictor response was somewhat more pronounced in the latter, especially at high stimulation rates, indicating an inhibitory β-adrenergic microvascular resistance effect of neural excitation in the intact vascular bed (lower curve). It can be seen from the figure that the myogenic microvascular constrictor response to the dynamic test stimulus was about 13 PRU in the control period (0 Hz) and that it was of almost the same amplitude at all rates of sympathetic stimulation in the intact vascular bed, but significantly increased when the β-receptors were blocked. The latter gradual increase of the dynamic constrictor response most likely can be explained by a physical gain effect of the successively raised sympathetic vascular tone prior to the superimposed dynamic stimulus (see Discussion). Since the neural α-adrenergic influence can be considered equal in the two series of experiments, the depicted significantly smaller dynamic constrictor responses in the intact vascular bed must be ascribed to a neural β-adrenergic inhibitory influence on myogenic reactivity.

In one cat the above described sympathetic stimulation experiments were performed after effective cholinergic blockade by atropine to eliminate possible influence of the sympathetic vasodilator fibres. The results obtained did not differ from those described, a finding compatible with the observation that only the relatively large proximal arterial vessels, but not the microvessels, in skeletal muscle are innervated by the sympathetic cholinergic fibres (Folkow, Mellander & Öberg 1961, Bolme & Fuxe 1967).

It could be argued that some of the reported resistance effects, especially at low flow as during intense sympathetic activation, could be explained by rheological effects of plasma fluid loss during the brief period of raised transmural pressure for test of dynamic reactivity. This possibility seems to be refuted by the following experiment. In two cats the vascular bed was paralysed with papaverine and flow was mechanically reduced to the same level as during nerve stimulation at 12 Hz. The test transmural pressure stimulus caused in this case no significant resistance increase.

Influence of adrenaline infusion. Adrenaline was administered by close arterial infusion to the sympathectomized muscle region in increasing amounts up to 2.7 μg kg⁻¹ min⁻¹. The experimental procedures to test dynamic myogenic reactivity were essentially the same as described above for sympathetic nerve stimulation.

The average results from observations on six cats before and after regional β-adrenergic blockade are illustrated in Fig. 3 in the two curves to the right (open and closed circles). The results are presented in a way analogous to that in Fig. 2. Points denoted C on each curve refer to the control situation before adrenaline and the four following points to effects obtained at gradually increasing adrenaline infusion rates. On the intact vascular bed, adrenaline infusion at the lowest rate (0.45 μg kg⁻¹ min⁻¹) tended to slightly decrease microvascular tone, but it was moderately increased at higher rates. After β-adrenergic blockade adrenaline, at the same infusion rates, always evoked microvascular constrictions, which were clearly more pronounced than in the intact vascular bed. This difference can mainly be ascribed to the competitive effect of β-adrenergic dilation and α-adrenergic constriction of adrenaline on the intact vascular bed. The superimposed dynamic microvascular constrictor response to the dynamic test stimulus, which in the control period was about 12 PRU, was significantly reduced by adrenaline on the intact

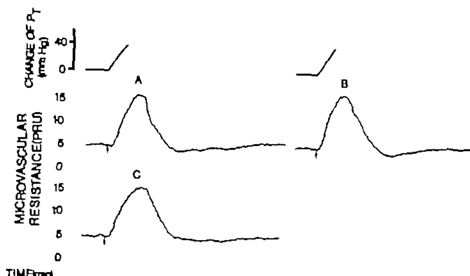


Fig 1 Myogenic microvascular resistance responses in sympathectomized skeletal muscle to a standardized transmural pressure (P_T) increase of 40 mmHg applied at a constant rate of 2.5 mmHg/s Panel A shows the response elicited 1 h after finished surgical preparation (control response) panel B the corresponding one elicited after an additional resting period of 30 min and panel C the response after effective α- and β-adrenergic blockade Note the pronounced constriction during the period of phasic increase in P_T (dynamic myogenic response) the much smaller constriction during the period of constant increased P_T (static myogenic response) and the fact that the amplitude of the response was of almost the same magnitude in all three cases

vascular constrictor effects were reflected as a simultaneous increase in overall vascular resistance in the muscle region and further that the more proximal arterial resistance vessels (i d >20–25 μm) and the large veins were largely irresponsive to dynamic myogenic stimuli It follows from these findings and from the comments on the technique used (see Methods) that the described resistance effects are caused by *active myogenic constrictions* in the microvessels in the studied muscle region

In the experiments to be described dynamic myogenic microvascular reactivity to the dynamic test stimulus was compared under control conditions and under the influence of adrenergic stimuli on the vascular bed The comparative results obtained under these different conditions will be expressed in terms of effects on the amplitude of the peak dynamic constrictor response Overall vascular resistance in the muscle region averaged 13.5±1 0 PRU in the control situation for the whole material

Influence of regional sympathetic nerve stimulation This type of experiment was performed in the following way Myogenic reactivity to the dynamic test stimulus was first determined in the control period (as exemplified in Fig 1 A) after which vascular transmural pressure was returned to th control level When control microvascular ton was re-established the regional sympathetic nerve were stimulated at a given frequency When steady state sympathetic microvascular constricto response was obtained the same dynamic te stimulus was again applied and this was done earl in the steady-state to minimize possible metabol dilator influence The dynamic constrictor respons to the test stimulus was permitted to be fully de veloped after which transmural pressure wa returned to the control level and the sympathetic stimulation turned off The same experimental pro cedures though with successively increasing sym pathetic stimulation rates in the range up to 12 Hz were repeated at intervals permitting return of microvascular tone to the control level during the recovery period between the experimental interventions Dynamic myogenic vascular reactivity was tested in this way at 6 different sympathetic excitation rates (see Fig 2) first in the vascular bed with intact adrenoceptors (below denoted 'intact vascular bed') and then after effective regional β-adrenergic blockade Such a series of experiments before and after β-blockade was performed on six different animals

in the α-blocked vascular bed (Fig. 3). The doses required to produce such β-adrenergic inhibitory effects were smaller for isoproterenol than adrenaline, complete inhibition being obtained with 1.4 μg/kg min⁻¹.

DISCUSSION

This study first confirms in essential respects previous observations by Viveros, Garlick & Renkin (1968) and especially those by Lundvall & Järhult (1976) of the presence of a β-adrenergic dilator component in the sympathetic neural control of microvascular tone in skeletal muscle, an effect which significantly can attenuate the simultaneous α-adrenergic constrictor component. A corresponding though more marked β-adrenergic dilator component was also observed during close arterial infusion of adrenaline. The findings by Lundvall et al. (1976, 1978a, 1978b) of neural and humoral β-adrenergic resetting of the ratio of pre-/postcapillary resistance and dilation of precapillary sphincters strongly suggested this β-adrenergic control mechanism to be involved in the regulation of transcapillary fluid exchange. The specific aim of the present study was to investigate the interaction between the adrenergic and the myogenic vascular control systems in the muscle microcirculation. The latter control system was analysed with regard to its recently described prominent dynamic component evoked in response to phasic increase in vascular transmural pressure (see Introduction). This dynamic component seems to be functionally important not only for transient myogenic responses but also for the establishment of normal basal myogenic tone (Grände & Borgström 1978b, Grände et al. 1979). The present results, taken together, demonstrated that sympathetic nerve activation, via a β-adrenergic component, exerted an effective inhibitory action on dynamic myogenic microvascular reactivity, graded in relation to the nerve excitation rate. Such graded β-adrenergic inhibitory effects were also revealed during infusion of adrenaline and noradrenaline, where adrenaline was shown to be the more effective *inhibitor*. A previous study (Grände & Mellander 1979) indicated that the static myogenic response to constant increased transmural pressure also was inhibited by β-adrenergic stimuli, suggesting that the described pattern of interaction might apply to myogenic control in general.

The hypothesis will be advanced below that the β-adrenergic component in sympathetic neural control might serve the seemingly important function to correct for nonlinearity in integrated adrenergic and myogenic peripheral resistance regulation, a phenomenon which seems to be a physical consequence of the law of Poiseuille.

The dynamic myogenic microvascular resistance response to a standardized transmural pressure stimulus was shown to be of roughly the same magnitude in the control period as during sympathetic nerve stimulation in the vascular bed with intact adrenoceptors, but significantly and gradually increased during nerve excitation after β-adrenergic blockade (Fig. 2). An analogous phenomenon after β-blockade was also revealed during catecholamine infusion (Fig. 3, right upper curve). This effect might at first glance be interpreted to indicate an α-adrenergic reinforcing effect on myogenic reactivity, but arguments will be presented below that it can rather be explained by a purely physical effect inherent in the gradual increase in adrenergic vascular tone prior to the dynamic stimulus.

It has been clearly established, for instance by Myers & Honig (1969), that the magnitude of a constrictor response in the resistance vessels to a given excitatory (e.g. vasomotor) stimulus is increased with increasing level of the vascular tone prevailing prior to the stimulus. This reinforcing phenomenon must be a factor that significantly adds to the complexity in overall vascular control, implying nonlinearity in peripheral blood flow and resistance regulation and obvious difficulties in predicting the effector response to a given stimulus in situations with differing vascular tone.

An attempt to elucidate the mechanisms behind *this* reinforcing phenomenon was made in a recent study (Borgström & Grände 1979) by using a mathematical approach to the investigation of dynamic microvascular reactivity to a given transmural pressure stimulus. In brief, this study showed that the phenomenon, with regard to the dynamic myogenic constrictor response, was somewhat influenced by the prevailing proximal arterial vessel resistance via its influence on intravascular pressure in the microvessels. It could, however, mainly be ascribed to an increase in gain implicit in the inverse fourth power relationship between microvascular resistance and internal vessel radius according to Poiseuille's law, though counteracted to

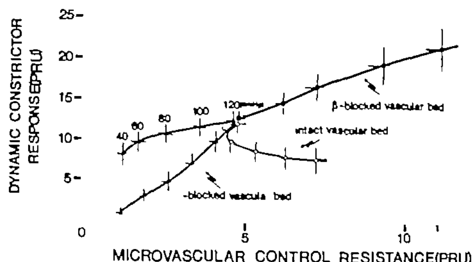


Fig 3 Curves to the right (open and cl sed circles) Effects of graded close arterial adrenaline infusions on microvascular resistance (abscissa) and on the superimposed dynamic myogenic constrictor response (ordinate) to a standardized test transmural pressure stimulus on the intact and β-blocked muscle vascular bed *Left lo er curve (closed triangles)* Corresponding effects after selective α-adrenergic blockade *Left upper curve (open triangles)* Effects on microvascular resistance (abscissa) and on dynamic myogenic reactivity (ordinate) to the test transmural pressure stimulus during graded decrease of arterial inflow pressure from 170 to 40 mmHg Points 'C' refer to the control situation in the 4 series of experiments

vascular bed but reinforced above control after β blockade The latter phenomenon is apparently analogous to that described for nerve stimulation and will be considered in Discussion The main conclusion is drawn that myogenic reactivity is clearly inhibited by the β adrenergic effect of adrenaline on the intact vascular bed

This conclusion was further corroborated by the results illustrated by the left lower curve in Fig 3 (8 cats) where dynamic microvascular reactivity was analysed during adrenaline infusion after α adrenergic blockade Under these circumstances the β-stimulating effect of adrenaline in increasing amounts caused a gradual decrease of microvascular tone towards virtually complete abolition (see abscissa Fig 3) Dynamic microvascular reactivity showed a parallel marked decrease interpreted to be caused mainly by a β adrenergic inhibitory influence of adrenaline. It might be argued that the effect of decreasing microvascular tone per se could explain the decreasing myogenic responses but this is apparently not a major cause as indirectly evidenced by the data in the left upper curve of Fig 3 This curve refers to experiments ($n=8$) in which microvascular tone was decreased over the same range as during adrenaline infusion but in this case evoked by an entirely different mechanism viz by regional reduction of arterial inflow pressure (see Method) Myogenic reactivity to the dynamic test stimulus (applied shortly after the pressure decline to minimize possible metabolic dilator influence) was in this case quite well preserved compared to the experiments during adrenaline infusion after α-blockade

Influence of noradrenaline infusion Noradrenaline infusions were performed on two cats in experiments analogous to those described for adrenaline The conclusions from these limited observations were in essential respects in agreement with those described for sympathetic nerve stimulation in so far that the dynamic myogenic responses were virtually unchanged by noradrenaline in the intact but increased in the β-blocked vascular bed (cf Fig 2) Inhibition of dynamic reactivity after α-blockade was observed during noradrenaline like adrenaline infusion though the effect for a given dose of the agents was quantitatively less pronounced for noradrenaline

Influence of isoproterenol infusion Such infusions at graded rates were performed in the intact vascular bed of two cats This drug decreased microvascular tone and the superimposed dynamic myogenic constrictor response in essentially the same manner as depicted for adrenaline infusions

n the α-blocked vascular bed (Fig. 3). The doses required to produce such β-adrenergic inhibitory effects were smaller for isoproterenol than adrenaline complete inhibition being obtained with 1.4 μg kg min

DISCUSSION

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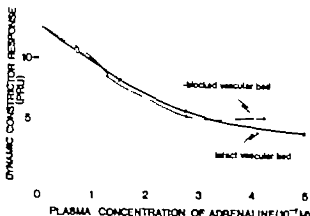


Fig 4 Mean values for dynamic microvascular constrictor responses to the test transmural pressure stimulus after correction for the physical gain effect of raised microvascular tone and the opposite effect during lowered tone plotted for graded infusions of adrenaline on the intact (open circles) and α-blocked (triangles) vascular bed *Data derived from the same experiments as depicted* in Fig 3

some extent by concomitant effects of a shift along the smooth muscle length tension curve and of the effect of Laplace law for thick walled vessels

The data presented in Fig 2 and 3 were specifically analysed with the use of the above-mentioned mathematical model (Borgström & Grände 1979 1980) The results of this analysis showed that the gradually increasing dynamic myogenic responses with increasing microvascular tone in the β blocked vascular bed during sympathetic nerve stimulation and adrenaline infusion could simply be explained by the net effect of the above-mentioned physical factors operating in vascular control This analysis thus strongly suggested that the increased myogenic responses during adrenergic influence on the β blocked vascular bed (Figs 2 and 3) could be explained without invoking any reinforcement of dynamic reactivity by α-adrenoceptor stimulation

This conclusion seems to be further supported by the data presented in Fig 4 where an attempt was made to deduce the true β-adrenoceptor influence of adrenaline on dynamic myogenic reactivity by correcting for the assumed physical gain effect of raised vascular tone on the magnitude of a superimposed constrictor response and for the opposite effect during lowered tone The increase in the dynamic responses above control as recorded on the β-blocked vascular bed (right upper curve in Fig 3) and the decrease in the dynamic responses below control at lowered arterial pressure (left upper curve in Fig 3) were then assumed to represent the mentioned physical effects of changed microvascular tone per se The myogenic responses during adrenaline infusion on the intact and the α-blocked vascular bed (Fig 3) were thus corrected for in Fig 4 by these values and here plotted versus the plasma concentration of adrenaline to take the varying blood flows into account It can be seen that with such correction dynamic reactivity was clearly inhibited during graded infusions of adrenaline and roughly to the same extent on the intact and the α blocked vascular bed The latter finding *that the two curves approach each other* seems to be compatible with the view that there is no prominent α-adrenoceptor mediated reinforcement of dynamic myogenic reactivity

The considerations in the foregoing point out some of the complexities in peripheral circulatory control where regulatory mechanisms act via influences on the circumferentially oriented smooth *muscle cells in the blood vessels As regards the* myogenic control system it would seem reasonable that its primary stimulus in all likelihood the transmural pressure change should result in a net effector response (e g blood flow or resistance change) which is directly proportional to the size of the primary stimulus irrespective of the prevailing vascular tone Yet the radius factor in Poiseuille s law per se inevitably leads to vast deviation from linearity in circulatory resistance control even if partially compensated for by the mentioned negative feed-back mechanism inherent in Laplace law and probably also by local accumulation of vasodilator metabolites as blood flow decreases. These latter effects however do not suffice to make the magnitude of the myogenic response independent of the prevailing vascular tone (see data in Fig 2 and 3 for the β-blocked vascular bed) but only provide coarse adjustment It seems that the β adrenergic mechanism *in neural (and humoral* noradrenergic) sympathetic control (Fig 2 lower curve) serves the important function of fine adjustment making the myogenic control system virtually linear implying the release of an almost equally large dynamic myogenic resistance response to a given primary transmural pressure stimulus irrespective of the prevailing level of vascular tone

The β-adrenergic influence of adrenaline was shown to be even more effective in modifying myogenic reactivity causing an actual decrease in the myogenic response *below the control level)* despite concomitant increase in microvascular tone (Fig 3

right lower curve). Such effects of adrenaline might be encountered in vivo only under relatively severe stress, for instance after hemorrhage (cf Grände & Mellander 1979). Effective inhibition of microvascular myogenic reactivity might under such circumstances be beneficial in so far it might improve capillary exchange by decrease in resistance and increase in functional capillary surface area (see Lundvall & Hillman 1978) in states of reduced blood flow

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Potentiation of the mechanical behavior of the human skeletal muscle through prestretching

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BOSCO, C. & KOMI, P. V. Potentiation of the mechanical behavior of the human skeletal muscle through prestretching. Acta Physiol Scand 1979. 106: 467–472. Received 16 Feb. 1979. ISSN 0001-6772. Kinesiology Laboratory, Department of Biology of Physical Activity, University of Jyväskylä, Finland.

Force-velocity and power-velocity curves in a vertical jump involving movements around several joints were derived from vertical ground reaction forces and knee angular velocities. The jumps were performed with weights from 10 to 160 kg added on the shoulders. The obtained curves from a semi-squatting static starting position resembled those reported for isolated muscles or single muscle groups. Vertical jumps were also performed in the conditions where the shortening of the leg extensors was preceded by prestretching of the active muscles either through preparatory counter-movement or dropping down on the force-platform from the various heights ranging from 20 to 100 cm. Prestretching modified through range of velocities the force-velocity and power-velocity curves by increasing both the ground reaction forces and the calculated mechanical power. Thus the results are similar to those reported in isolated muscles. In studies with isolated muscle preparation the nervous connections have not been intact and therefor it is suggested that increase in the performance of the skeletal muscles through prestretching, in the conditions of the present study, was attributed to the combined effects of the utilization of stored elastic energy and the reflex potentiation of muscle activation.

Key words: Muscle mechanics, elastic energy, muscle activation

The elastic properties of the muscle play an important role in determining the mechanical behavior of skeletal muscle. Cavagna et al. (1965) have pointed out that stretching of an activated muscle leads to a greater work and power output during the shortening phase of contraction. Recently Cavagna & Citterio (1974) working on isolated frog striated muscle have pointed out that the force-velocity relationship is changed if the muscle was released immediately after stretching.

In the present study an attempt is made to observe if the force velocity and power-velocity curves, derived from vertical ground reaction forces and knee joint angular velocities, could be modified through prestretching, in conditions where the pattern of movement is natural and integrated by the central nervous system.

METHODS

A 22-year-old male volley ball player (mass 84 kg and height 187 cm) performed vertical jumps on a force platform with the following three different initial starting positions:

(1) Semi-squatting position in which no preparatory counter-movement was allowed. From this starting position a series of 8 jumps was performed with barbell weights on the shoulders. The eight vertical jumps consisted of performances with the loads of 10, 20, 40, 60, 80, 100, 110 and 160 kg. In order to begin the vertical jumps the subject flexed the knee until he felt a comfortable starting position. This occurred normally at a knee angle of approximately 85°. From this starting position the subject performed maximal vertical jumps and landed on the force-platform with the legs kept straight. An additional jump was performed from the same starting position without load. All these jumps were called static jumps (SJ).

(2) Jumping with a preparatory counter-movement. In this counter-movement jump (CMJ) the subject started from erect standing. The downward counter-movement was approximately to the same knee angle as the starting position in SJ. The highest load (160 kp) was not used in CMJ.

(3) Jumping after different stretch loads were given to the active leg extensor muscles by letting the subject drop himself on to the platform from heights of 20, 40, 60, 80 and 100 cm. No additional loads were used in these drop jumps (DJ). Two variations of DJ were investigated. In

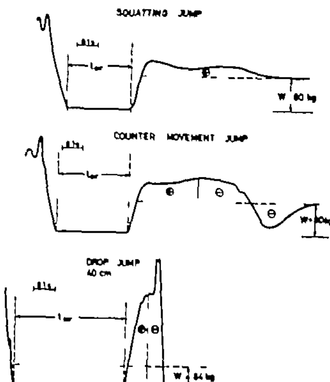


Fig 1 Examples of the force-time curves of the vertical ground reaction forces in the different jumping conditions Squatting Jump Counter Movement Jump and Dropping Jump Symbols. W=weight of the subject ⊖=phase of deceleration in eccentric (negative) work ⊕=phase of concentric (positive) work t_{air}=phase when the jumper is in the air Landing on the platform terminates this phase Movement of the recorder is from right to left

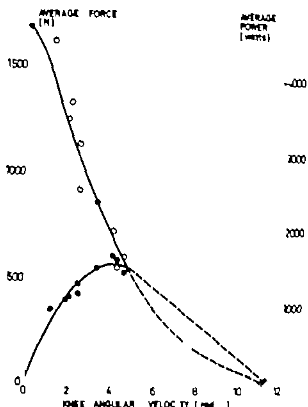


Fig 2 Force-velocity (○) and power velocity (●) for the vertical jump performed from a static starting position (squatting jump)

one condition the subject performed the vertical jumps immediately after the touch down on the platform The change in knee angle during contact was consequently small In the second condition the subject was allowed to damp the gravitational pull longer with a greater change in the knee angle

Maximal isometric force with zero angular velocity at all joints was also measured on the force-platform In this test the subject was in the same position as when starting the SJ The bar on the shoulders was fixed at both ends thus allowing no movements in the joints

Angular velocity and amplitude of movement around the knee joint were recorded with an electrogoniometer which was calibrated to read zero degrees when the subject was standing erect Electrogoniometry was also used to obtain the *maximum speed of knee extension* In this test the subject was lying in a supine position with the knees flexed to a 90° starting position

Instrumentation and mechanical calculation

In order to record the ground reaction forces a force-platform of Komi et al. (1974) was used Examples of the vertical force-time curves are shown in Fig 1

The force-time curve and the flight time gave the basis for calculation of the following mechanical parameters vertical velocity at take-off (V_v) average force ($\bar{F}$) and average mechanical power ($\bar{W}$)

The vertical velocity at take-off was obtained from the formula.

$$V_v = \tfrac{1}{2} \times t_{air} \times g \quad (1)$$

in which g=acceleration of gravity (9 81 m/s²)

Average force ($\bar{F}$) was computed with the following formula

$$\bar{F} = \frac{m \times V_v}{t} \quad (2)$$

in which t=the respective time of the force-time curve m=mass of the subject V_v=vertical velocity at take off

The average mechanical power ($\bar{W}$) was computed through double integration of acceleration calculated from the force-time curve on the Hewlett Packard Model 9810 A desk computer This computation gave the total mechanical work (W) which was then divided by the respective total time to obtain ($\bar{W}$)

In DJ condition at the moment of touch down on the force-platform the ground reaction force reads positive value although the work is negative (eccentric phase) as denoted in Fig 1C In order to estimate the periods of negative and positive phases from the total contact period

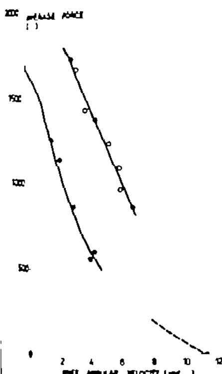


Fig. 3. Relationship between the average force and the knee angular velocity in two different conditions of the vertical jump. Squatting Jump (●), Counter Movement Jump (O).

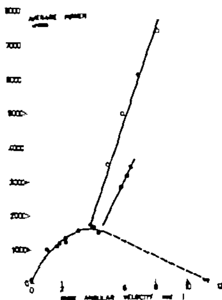


Fig. 4. Power velocity curves for three different conditions of the vertical jump: Squatting jump (●). Dropping Jump with damped touch down before take off (O). Dropping Jump with undamped touch down before take off (□).

the negative contact time was first calculated by using the formula of Asmussen & Bonde-Petersen (1974) as follows:
Negative contact time

$$t_{neg} = \frac{V_d \cdot t_{tot}}{V + V_d} \quad (3)$$

In which V = vertical take-off velocity, t_{tot} = total contact time, V_d = downward vertical velocity.
V_d can be obtained from the formula:

$$V_d = \sqrt{2gh}$$

In which h = dropping height, g = acceleration of gravity 9.81 m/s².

RESULTS

In the squatting jump (SJ) the force-velocity curve of Fig. 2 was obtained. The maximal values of isometric force and knee angular velocity were 1688 N and 11 24 rad/s respectively. Fig. shows also an experimental curve of the average mechanical power ($\bar{W}$) for the different knee angular velocity values. The average maximal power was 1672 watts. This was produced when the average force $\bar{F}$ was about 40% of isometric maximum, and when the speed of shortening of the extensor leg muscle reached 35% of the maximal angular knee extension velocity.

Fig. 3 compares the force-velocity curves between SJ and counter-movement jump (CMJ). As is evident from the figure the velocity of knee extension at any given load was always higher in the CMJ.

The experimental curves of the average power output for the SJ and drop jump (DJ) are shown in Fig. 4. In the DJ-conditions, when the subject did not damp the gravitational pull upon the touch down (upper curve in Fig. 4) the power curve was at a higher level as compared to the condition in which damping was allowed. A similar comparison of the force-velocity curves of the damped and undamped DJ is presented in Fig. 5.

DISCUSSION

The total body vertical jumping is a multi-joint movement, which is some function of the combined

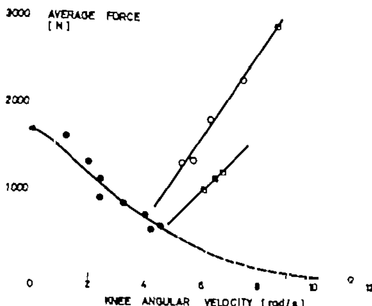


Fig 5 Force-velocity curves for three different conditions of the vertical jump. Squat ting Jump (●) Dropping Jump with damped touch down before take off (○) Dropping Jump with undamped touch down before take off (□)

impulses of all muscles participating in the movement. If during the phase of the movement from which force and velocity calculations are made one muscle group is dominant for example quadriceps it is reasonable to assume that the ground reaction force time curve is related to the quadriceps muscle group forces and that the angular velocity of the knee joint is related to the linear velocity of shortening of that muscle group Allowing these assumptions it is thus possible to relate the curves of Figs 2, 3 4 and 5 to force-velocity and power velocity curves reported on isolated muscle by Hill (1938) and on single muscle groups by Asmussen et al (1965) and Komi (1973) Since the preparation of this report several other subjects have been measured on the conditions comparable to those of the present study Although the analysis was not performed in the same detail the results were in principle similar to the ones reported here

The results obtained in the different conditions of the study suggest first that the shift in the force-velocity curve to the right (Fig 3) in the counter movement jump reflects the storage of elastic energy and its utilization by the leg extensor muscle in this kind of performance Secondly when the vertical jump is performed immediately after dropping down from various heights (DJ-condition) the muscles in concern perform mechanical work mainly through a spring-like behavior in which the restitution of elastic energy plays an important role

Explanations for these observations can be sought from the basic information about the elastic behavior of skeletal muscle under various experimental conditions As is known from the experiments on isolated muscle (Cavagna et al 1965) an activated muscle can store a substantial amount of elastic energy when it is forcibly stretched This stored energy can in turn be recovered at least in part in the following concentric work phase provided that the time lag within the stretch-shortening cycle is short In fact the present result is in good agreement with similar findings in the work velocity curve of an isolated muscle (Cavagna et al 1968) In isolated muscle preparations all the nervous connections are cut and therefore the potentiation of the performance after the prestretching must be attributed solely to the storage and utilization of elastic energy and their influence on contractile machinery

The present experiment was performed with a normal human subject and consequently the nervous connections were intact Therefore it is likely that the nervous system also interfered concomitantly with the elasticity phenomenon There are two factors which should be pointed out here First the leg extensor muscles are active before the contact on the ground in hopping movements (Melvill-Jones & Watt 1971) and also before the eccentric contraction phase in counter movement jumps (Desupres 1976) In both cases the activation occurs

only enough to exceed the average electromechanical delay of 50 ms (Komi & Cavanagh 1977), and thus prepares the muscles to resist the gravitational pull. In order to store elastic energy the muscles must be activated during the stretch (Cavagna 1968). Secondly this preactivation period is also long enough to allow the possible potentiation of alfa motor neuron activation via the stretch reflex. In fact, evidence has been presented which shows that in moderately fast running the electromyographic activity of the gastrocnemius muscle during the stance phase exceeds the level recorded during maximal voluntary isometric contraction (Dietz et al. 1978). However at the present stage of the studies in progress, it is not possible to state the relative role of the two factors—mechanical and reflex—in potentiating the jumping performance. It must also be emphasized that in order to maintain a steady starting position there is some preactivation of the leg extensor muscles even in the squatting jump. According to the basic function of the muscle spindle (Matthews 1972) the length sensitive secondary afferents are probably activated in a starting position of the SJ but in counter-movement or drop jumps the primary afferents could be the ones responsible for the reflex potentiation of the activity. Thus it is possible that differences exist in the reflex control of the various jumps, which would then result in differences in the force generation.

In the "damped" and "undamped" drop jump conditions both the force-velocity and the power-velocity curves were different (Figs. 4 and 5). When the drop jump was performed with no delay on the ground (undamped) the power and force values were higher than when the damping movement was allowed. These differences could perhaps be explained partly by the concept of "short range stiffness" introduced by Rack & Westbury (1974). This concept implies that the muscle performs like a spring when the length change during stretch is very short. The short range stiffness can also be thought to reflect the combined stiffness of the crosslinks between actin and myosin filaments at the moment of contact on the ground. Hill (1970) has suggested that the contractile machinery is "locked" in a strained condition. Increasing the range of stretch (Rack & Westbury 1974)—in this study the amplitude of the knee angle change upon touch down—is likely to decrease the elastic behavior of the muscle. When damping was allowed in the drop jumps the possible effect of the short range stiffness disappeared and part of the stored elastic energy was dissipated as heat. Consequently it could not be utilized in the subsequent concentric phase of the contraction. Cavagna et al. (1972) have also reported that increasing the amplitude of the movement during contact decreases the utilization of the stored elastic energy.

In summary the following observations are significant from this study: (1) the force-velocity and power-velocity curves, measured under normal movement conditions in which several joints are involved, resembles those in isolated muscles (Hill 1938) or in human movements when one muscle group was involved with either variable speed (Wilkie 1950) or constant speed (Asmussen et al. 1965, Komi 1973). (2) Both the average ground reaction force and the calculated mechanical power through a range of velocities are enhanced when the vertical jump is performed with a preliminary counter-movement which allows the prestretching of active muscles. The increase in performance is attributed to a combination of the utilization of elastic energy and to the stretch reflex potentiation of the muscle activation at least until such time as the two possible mechanisms can be separated experimentally.

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Effect of sensitization on spontaneous and phosphatidylserine-induced histamine release and on cyclic AMP and GMP levels in isolated rat mast cells

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Sprague Dawley rats were sensitized with 20 µg or 100 mg egg albumin (using pertussis vaccine as adjuvant). Mast cells isolated from the former group of animals showed a higher degree of histamine release upon challenge in vitro with egg albumin than those from the latter group. Using the lower amount of antigen for immunization mast cells from Hooded Lister rats showed an even higher degree of histamine release induced by antigen An increased antigen-induced histamine release was associated with an increased spontaneous and phosphatidylserine-induced histamine release. Histamine release induced by phosphatidylserine was found to be specific in so far as it was calcium dependent and theophylline-inhibited. The basal level of cyclic AMP in mast cells was significantly depressed by sensitization There was a relationship between the cyclic AMP/cyclic GMP ratio and the degree of spontaneous, phosphatidylserine-induced and anaphylactic histamine release. The results suggest that sensitization induces an increased release of histamine not only to the specific antigenic stimulus but also to more unspecific stimuli Concomitantly there is fall in the cyclic AMP/cyclic GMP ratio The relationship between these two phenomena is discussed

Key words: Anaphylactic histamine release immunization, inbred Hooded Lister rats, Sprague Dawley rats, strain differences

It is notoriously difficult to obtain well sensitized rat mast cells for studies of anaphylactic histamine release. The commonly used Sprague Dawley rats have low levels of IgE (Bennich et al. 1976) This may be one reason for low and variable sensitization. A second factor could be the amount of antigen used for sensitization (Jarrett & Stewart 1974) Very high amounts of antigen such as those previously used in this laboratory (e.g. Sydbom & Uvnäs 1976), may not only fail t provoke antibody synthesis but can even establish a state of specific unresponsiveness (see Ersen 1974) To complicate matters further the responsiveness to antigen may deteriorate during the isolation of mast cells (Johnson & Moran 1966, Norn 1968).

In the present study we have therefore investigated the influence of the amount of antigen used in the sensitization procedure as well as the possibility that a high IgE producing strain of rats may give better sensitized mast cells. For the latter purpose Hooded Lister rats were used since these rats rank high as IgE producers (Bennich et al 1976) Elsewhere the relationship between circulating IgE levels and antigen-induced histamine release from isolated mast cells will be reported (Sydbom & Karlsson 1979)

During the course of our studies with anaphylactic histamine release we have also noted that spontaneous histamine release tends to be higher in mast cells from sensitized animals We also report that phosphatidylserine a compound, used to enhance antigen induced histamine release (Goth et al 1971) per se increased histamine release and that this effect is enhanced in mast cells from sensitized rats

Finally in view of the suggested role of cyclic

nucleotides as mediators or modulators of histamine release (Orange et al 1971 Kaliner & Austen 1974) we have studied the influence of sensitization on the concentration of cyclic AMP and cyclic GMP in mast cells

MATERIAL AND METHODS

Egg albumin was obtained from Difco Laboratories Detroit, Michigan USA Ficoll from AB Pharmacia, Uppsala, Sweden human serum albumin from AB Kabi Stockholm Sweden and phosphatidylserine from Koch Light Laboratories Colnbrook Bucks England Pertussis vaccine (lot no 120274) was obtained from Statens seruminstitut Copenhagen Denmark 2-(^{3}H) cyclic adenosine 3 ,5-monophosphate (26 Ci/mmol) was obtained from the Radiochemical Centre, Amersham England Cyclic AMP binding protein was prepared as described by Brown et al (1972) (^{125}I)-tyrosyl-succinyl adenosine 3 ,5 monophosphate (60–90 Ci/mmol) and (^{125}I)-tyrosyl-succinyl guanosine 3 ,5 -monophosphate (100–140 Ci/mmol) was obtained from New England Nuclear Chicago USA The cyclic AMP antibodies used were either those kindly given to us by Dr G Brooker or those commercially obtained from New England Nuclear The cyclic GMP antibodies were either obtained from New England Nuclear or those prepared by ourselves as described previously (Fredholm Belfrage & Blaschke 1977)

All other chemicals were obtained from the usual commercial sources The Hooded Lister rats ("Inbred IgE") were originating from rats purchased from Möllegaard Avlslab ApS L. Skensved Denmark and inbred and reared by Dr T Karlsson.

Sensitization of rats

With high dose of antigen Male Sprague Dawley rats (180–200 g) were injected s.c. (in the abdominal skin) with 2×0.5 ml of egg albumin (100 mg/ml) and in the neck with 2×0.5 ml of pertussis vaccine as an adjuvant

With low dose of antigen Male Sprague Dawley and Hooded Lister rats were injected s c (in the abdominal skin) with 2×0.5 ml of pertussis vaccine in which 20 µg/ml egg albumin was dissolved

Isolation of mast cells

Mast cells were usually taken from the rats 2 to 4 weeks after the injections Mast cells were collected only from the pleural cavities of the rats, pleural mast cells have been found to become more sensitized than peritoneal cells (Sydbom & Uvnäs 1976) and isolated essentially as described by Thon & Uvnäs (1976) After separation by gradient centrifugation on Ficoll (350×g) the cells were washed 3 times at 20°C with an isotonic saline solution containing 10% (v/v) Sörensen s phosphate buffer (Na_2HPO_4 + KH_2PO_4 67 mM) pH 6.3 containing 1 mg/ml human serum albumin.

Incubation procedure

The isolated mast cells (about 5000 cells/ml) were incubated in 2 ml isotonic saline solution (NaCl 137 mM KCl 2 7 mM $CaCl_2$ 1 8 mM $MgCl_2$ 1 mM) containing 10% (v/v) Sörensen s phosphate buffer (67 mM) pH 6.7 and human serum albumin (0.5 mg/ml) Egg albumin as antigen was added in a concentration of 10 µg/ml for 10 min at 37°C Phosphatidylserine was dissolved in a small volume of saline solution (without albumin) and sonicated for 1 min at 0°C with an MSE ultrasonicator (8 microns) to get a good emulsion When phosphatidylserine was added to the incubation medium it was used in a final concentration of 5×10^{-5} M (40 µg/ml)

The incubation was terminated by immersing the tubes in ice water and then centrifuging (350×g) for 10 min at 4°C The supernatants were decanted off into new tubes and 0.2 ml of 0 1 M HCl was added to the cell residues. The supernatants and the cell residues were heated in a boiling water bath for 5 min 1 8 ml of saline solution was added to the cell residues to readjust the volume Histamine was determined in both supernatants and cell residues by the method of Shore et al. (1959) omitting the extraction procedure (Bergendorff & Uvnäs 1972). Histamine release was expressed as a percentage of total histamine. Unless otherwise stated the spontaneous histamine release has been deducted from the values presented Samples were run in duplicates.

Determination of cyclic nucleotides

To measure the basal levels of cyclic nucleotides mast cells were isolated separately from a number of rats. The mast cells were counted in a Bürcher chamber and suspended in 450 µl of basal incubation medium and allowed to stand for 10 min at 37°C The incubation was stopped by adding 50 µl of 100% TCA (Trichloroacetic acid) and placing the tubes in ice water The tubes were then stored at −20°C before testing for cyclic AMP and cyclic GMP Following acidification with 0.1 M HCl the TCA was removed by fourfold extraction into 10 volumes of ether The ether was subsequently removed by heating to 50°C Cyclic AMP was determined either by the competitive binding method of Brown et al (1972) or by radioimmunoassay (Steiner et al 1972) When the assay was performed with the former method cyclic AMP was purified as described previously (Fredholm et al 1976). The radioimmunoassay procedure included an acetylation step as described by Harper & Brooker (1975) Identical values were obtained with or without prior purification of the samples. Furthermore the values obtained were similar in undiluted samples and those diluted 1 3 1 30 and 1 70. The recovery of exogenous cyclic nucleotides added to the extracts before ether extraction was close to complete and no corrections for incomplete recovery were made Results are expressed as pmol cyclic nucleotide per 10^6 cell

RESULTS

Effect of antigen dose on sensitization Comparison between Sprague Dawley and Hooded Lister rats

Two groups of Sprague Dawley rats were sensitized with egg albumin In the first group 10 rats were injected with a rather large dose of antigen 100 mg

Table 1 *Histamine release from isolated mast cells from Sprague Dawley or Hooded Lister rats sensitized with different amounts of antigen*

Antigen (10 µg/ml) or phosphatidylserine (PS) (5×10^{-4} M) was incubated for 10 min at 37°C with the mast cells. The result expressed as per cent histamine release ± S.E. Number of rats in each group are given in brackets

	Sprague Dawley			Hooded Lister	
Sensitized with	Control	100 mg egg albumin	20 µg egg albumin	Control	20 µg egg albumin
Spontaneous release	5 4±0 4 (15)	4 7±0 5 (10)	5 0±0.3 (7)	7 0±0 4 (17)	8.7±0 5 (45)
				$P<0.05$	
Antigen (−spont. rel.)		2.6±0.6 (10)	9.5±1.8 (7)	–	18 4±1.2 (51)
		$P<0.001$		$P<0.01$	
PS (−spont. rel.)		4 2±0.8 (10)	9 6±2.3 (7)	5 1±0.4 (6)	11 7±1.0 (35)
		$P<0.05$		$P<0.05$	
Antigen+PS (−PS and spont. rel.)		15.2±2.4 (10)	25.3±4 7 (7)		17.5±1 4 (35)

egg albumin, a dose that we have used in previous studies (Sydbom & Uvnäs 1976) and in the second group 7 rats were injected with a very small dose of antigen, 20 µg egg albumin. Mast cells were isolated from the rats separately and incubated for 10 min at 37°C with buffer only (spontaneous release), with antigen 10 µg/ml, with phosphatidylserine 5×10^{-4} M or with antigen and phosphatidylserine simultaneously and the histamine release from the mast cells were determined (Table 1). The results show that antigen induced release from Sprague Dawley mast cells was significantly increased from 2.6 to 9.5% ($p<0.001$) when using small doses of egg albumin to sensitize the rats. However the release by phosphatidylserine per se was also significantly increased from 4 2 to 9.6% ($p<0.05$). When phosphatidylserine was added to the incubation medium to potentiate the effect of antigen, the histamine release was 15.2 and 25 3% respectively when using mast cells from rats sensitized with high doses (100 mg) or low doses (20 µg) of egg albumin. Thus when small doses of egg albumin were used for sensitization the mast cells were more sensitive as judged by antigen-induced histamine release compared to high doses of egg albumin.

Another strain of rats namely the Hooded Lister rats was tested as well. Based on the previous results the Hooded Lister rats were sensitized with the low dose of egg albumin (20 µg). The isolated mast cells were incubated with buffer antigen, phosphatidylserine or antigen plus phosphatidylserine like before. The histamine release was determined and the results were compared with those from Sprague Dawley rats sensitized with low doses of antigen (20 µg egg albumin) (Table 1). The histamine release after antigen challenge was significantly ($p<0.01$) higher from Hooded Lister mast cells (18 4%) than from Sprague Dewley mast cells (9.5%). The effect of phosphatidylserine alone also tended to increase

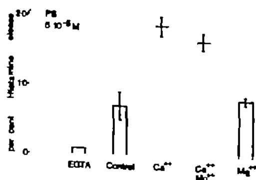


Fig. 1 Histamine release from mast cells isolated from highly sensitized Hooded Lister rats (20 µg egg albumin) Phosphatidylserine (PS), 5 10^{-4} M was incubated in saline with the mast cells for 10 min (control) or with Ca^{2+} (1.8 mM) Mg^{2+} (1 mM) or in the presence of 1 mM EDTA

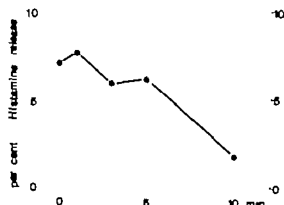


Fig 2 Histamine release from mast cells isolated from highly sensitized Hooded Lister rats (20 μg egg albumin) The mast cells were incubated in saline with 1 mM EGTA and 5×10^{-6} M phosphatidylserine (PS) and 2.8 mM Ca^{2+} was added to the medium at different times after phosphatidylserine at zero time and after 1 3 5 and 10 min respectively

Spontaneous histamine release from mast cells

The spontaneous histamine release from Sprague Dawley mast cells was 4 7 and 5 0% with the different sensitization techniques used (Table 1) and did not differ significantly from normal unsensitized Sprague Dawley mast cells (5 4%) However Hooded Lister mast cells showed an increased spontaneous release after sensitization from 7 0% for normal mast cells to 8 7% ($p<0$ 05) for sensitized mast cells

The effect of phosphatidylserine on histamine release from mast cells

Phosphatidylserine (5×10^{-6} M)-induced histamine release in isolated mast cells from normal Hooded Lister rats was 5 1±0 4 ($n=6$) and from mast cells from Hooded Lister rats sensitized with low dose of antigen (20 μg) it was 11 7±1 0% ($n=35$) where the spontaneous histamine release has been deducted (Table 1) The release was Ca^{2+} dependent since omitting Ca^{2+} from the incubation medium will inhibit the release by about 65% and with 1 mM EGTA in the medium the histamine release was almost completely blocked (Fig 1) There is also a "desensitizing" phenomenon If Ca^{2+} is added to the mast cells in the incubation medium after phosphatidylserine there was a decrease in the response and after 10 min delay the histamine release with phosphatidylserine is decreased to 25% of the initial value (Fig. 2) Finally the effect of phosphatidylserine on histamine release could be reduced by theophylline (2.5 mM) to about 40% of control In a series of expts histamine release with phosphatidylserine (5×10^{-6} M) was 8.7±2.6% ($n=6$) and when theophylline (2.5 mM) was present 3 4±1.3% ($n=6$)

Basal levels of cyclic nucleotides from normal and sensitized rats

The basal level of cyclic AMP in normal Sprague Dawley mast cells was 0 62±0 18 pmol per 10^6 cells (Table 2) which is close to the value previously reported by us (0 58±0 07 Fredholm et al 1976) The cyclic AMP level of mast cells from Hooded Lister rats were significantly ($p<0$ 01) lower in sensitized compared to non sensitized animals The cyclic GMP levels are rather similar in magnitude to the cyclic AMP levels The cyclic GMP level in mast cells from Hooded Lister rats is significantly ($p<0$ 01) higher than that in mast cells from Sprague Dawley rats but there was no effect of sensitization on the cyclic GMP of mast cells

DISCUSSION

The present results show that antigen-induced histamine release from isolated mast cells depends both on the strain of rats used and the amount of antigen injected during immunization Clearly the low dose of antigen gives a more satisfactory sensitization than the high one Moreover Hooded Lister rats show a more satisfactory response than Sprague Dawley rats The use of 20 μg egg albumin injected together with pertussis vaccine to Hooded

Table 2 *Basal levels of cyclic nucleotides in isolated rat mast cells from Hooded Lister rats normal or highly sensitized (20 μg egg albumin) and from Sprague Dawley rats*

The result is expressed as pmol/10^6 cells ± S E

Mast cells	cAMP (pmol/ 10^6 cells)	cGMP (pmol/ 10^6 cells)	cAMP/ cGMP
Hooded Lister normal ($n=12$)	0 97±0 15	0.32±0 04	3 03
Hooded Lister sensitized ($n=16$)	0 58±0.09	0.35±0 02	1 66
Sprague Dawley normal ($n=12$)	0 6_±0 18	0 16±0 03	3.88

Lister rats sacrificing the animals 2–4 weeks after immunization appears to produce sufficiently large and reproducible anaphylactic histamine release in vitro to obviate the need for a more systematic attempt to optimize the conditions of sensitization.

Interestingly the mast cells exhibiting a high degree of anaphylactic histamine release also had a higher spontaneous release and an increased histamine release following the addition of phosphatidylserine. The spontaneous histamine release is the release occurring in the absence of any specific, recognized stimulus. The spontaneous histamine release is not influenced by extracellular calcium ion concentration (Foreman 1973). It may at least in part reflect the spontaneous leakage of histamine from cells damaged during the isolation and incubation and hence not be dependent upon exocytotic release. An increased spontaneous histamine release may therefore reflect an increased fragility of mast cells. By contrast the present results show that histamine release induced by 5×10^{-4} M phosphatidylserine is dependent on the presence of extracellular calcium. Foreman & Mongar (1975) have proposed a model according to which antigen acts as a histamine releasing agent by virtue of opening calcium gates in the membrane. When antigen is administered to sensitized mast cells in the absence of calcium no histamine release occurs. However subsequent administration of calcium may provoke release provided that the latency between the antigen-antibody stimulus and the addition of calcium is kept small. These authors also show that this latency is prolonged in the presence of phosphatidylserine so that calcium may cause histamine release at a time when it would not have caused any release had not phosphatidylserine been present. They suggest that antigen acts by opening calcium gates and phosphatidylserine by maintaining the gates open for a longer period of time. The present results show that phosphatidylserine behaves similarly as does antigen. Thus according to the model of Foreman & Mongar (1975) phosphatidylserine may by itself open calcium gates. Phosphatidylserine did not induce any histamine release when extracellular calcium was removed by EGTA. However subsequent administration of calcium caused release of histamine which decreased with the time elapsing between phosphatidylserine and Ca^{++} administration. This agent thus seems to be the cause of a transient increase in the permeability to calcium, in agreement with the results obtained with antigen, but in contrast to those obtained with a calcium ionophore A 23 187 (Foreman & Mongar 1975).

There is evidence that anaphylactic histamine release from lung tissue is decreased when the tissue cyclic AMP level is increased by several different agents (Orange et al. 1971) and enhanced when it is decreased (Tauber et al. 1973, Kaliner et al. 1972). By contrast increased levels of cyclic GMP appears to enhance anaphylactic mediator release (Kaliner et al. 1972, Kaliner 1977). The situation is much less clear with regard to isolated mast cells. Kaliner & Austen (1974) reported that increases in mast cell cyclic AMP were associated with a decreased histamine release induced by reversed anaphylaxis. Sullivan & co-workers (1975) similarly found that compound 48/80 decreased mast cell cyclic AMP levels and that agents that inhibited the histamine release enhanced cyclic AMP. However a later study from the same laboratory showed that anti-IgE as well as concanavalin A actually enhance mast cell cyclic AMP levels (Sullivan et al. 1976). Furthermore neither Johnson et al. (1974) nor Fredholm et al. (1976) could demonstrate any clear relationship between cyclic AMP levels and histamine release. These apparently conflicting results in mast cells have been attributed to compartmentalization (Sullivan et al. 1976). In view of the apparently reciprocal effects of cyclic AMP and cyclic GMP in the lung it is possible that also in mast cells changes in the ratio of cyclic AMP and cyclic GMP would correlate better with histamine release than just changes in one of the nucleotides.

In the present study the level of cyclic GMP in mast cells is reported. It is quite close to the concentration of cyclic AMP so that the ratio cyclic AMP/cyclic GMP is between 1 and 5, in agreement with Ishizaka & co-workers (1975). The ratio was different between non-sensitized and sensitized mast cells, due to a significantly depressed cyclic AMP level in sensitized cells. It is interesting in this context that a significantly depressed cyclic AMP response to histamine is found in the sensitized guinea pig lung (Mathé et al. 1978). These authors discuss the possibility that the decreased cyclic AMP response to histamine is due to "desensitization" in response to increased availability of histamine. The fact that spontaneous leakage of inflammatory mediators increases in the lung after sensitization (Mathé et al. 1977) was taken to support this conclusion.

Thus we are faced with the situation that the mast cell cyclic AMP/cyclic GMP is decreased in parallel with an increased *spontaneous* and *phosphatidylserine* induced histamine release but we are unable to decide the cause and effect The concept developed by Kaliner Austen & co-workers would suggest that spontaneous histamine release is increased because *the cyclic AMP/cyclic GMP ratio* in mast cells is decreased Conversely the discussion and results of Mathé & co-workers (1978) could be used to argue that the cyclic AMP/cyclic GMP ratio is decreased because the spontaneous *histamine* release is increased *It* is of course also possible that the two phenomena are not directly causally related and that they are either totally unrelated or caused by some other change such as an alteration in cellular calcium metabolism It is a *well known fact that atopic patients* are hyperreactive to all kinds of non-immunological stimuli and that this hyperreactivity is correlated with the fluctuations of the disease The present results showing an increased sensitivity of mast cells from immunized animals could have a bearing on this clinical observation

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Shortening velocity active force and homogeneity of contraction during electrically evoked twitches in smooth muscle from rabbit urinary bladder

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UVELIUS B Shortening velocity active force and homogeneity of contraction during electrically evoked twitches in smooth muscle from rabbit urinary bladder Acta Physiol Scand 1979 106: 481–486. Received 6 March 1979 ISSN 0001-6772. Department of Physiology and Biophysics, University of Lund, Sweden.

Force-velocity relations obtained by the quick release technique were studied at different times during the twitch at 25°C in electrically stimulated longitudinal smooth muscle from rabbit urinary bladder The results show that maximal shortening velocity (V_{max}) and maximal tension (P_0) reach their highest values during the rising phase of the twitch. Maximum V_{max} was calculated to be 0.30±0.04 l/s (S.E. n=9). V_{max} declines rapidly with time and at the peak of the twitch it is only about 70% of the maximum value. On the other hand P_0 at the peak does not differ significantly from its maximum value during the rising phase. In the relaxation phase of the twitch, both V_{max} and P_0 decrease progressively Photographic studies of preparations marked with charcoal grains did not show any considerable inhomogeneity of contraction during isometric twitches maximum movement of grains never exceeding 4% of total muscle length. No yield at the ends of the preparations were seen The different time course of V_{max} and P_0 can therefore not be explained by longitudinal inhomogeneity of activation or deactivation of the preparations.

Key words Urinary bladder twitches force velocity homogeneity

The classical muscle model of Hill (1938) describes skeletal muscle mechanics in terms of two components (1) a contractile element which immediately adjusts its shortening velocity to a change in load in accordance with its characteristic force–velocity curve, and (2) an undamped series elastic element which immediately adjusts its length to a change in load. This model implies that an isometric contraction is associated with internal shortening of the contractile element and lengthening of the series elastic unit. In order to describe the chemomechanical transduction in the contractile machine Hill (1949) defined "intensity of the active state" as the force which the contractile component would generate if its length were held constant. According to these ideas the time course of the active state can be measured in contraction and relaxation by determination of tension during quick stretch (Hill 1949) or controlled release (Ritchie 1954) or of the capacity of the muscle to shorten against a small load (Jewell & Wilkie 1960) With increasing knowledge of the mechanism of excitation–contraction coupling, the active state was considered to give an indication of the amount of activator calcium bound to the contractile proteins (see e.g. Edman & Kiessling 1971). This "classical approach" to the study of the time course of activation has been applied to smooth muscle and the results resemble qualitatively those obtained with skeletal muscle (Gordon & Siegman 1971 Johansson 1973)

During recent years the concept of active state has been challenged mainly as a result of new studies on skeletal muscle (see e.g. Simmons & Jewell 1974 Julian & Moss 1976) A significant part of the elasticity of the contracted muscle is now considered not to lie directly in series with the contractile machinery but to be associated with the cross bridges themselves. Moreover the shortening

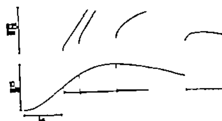


Fig 1 Superimposed registrations of quick releases to the same afterload (4 mN) early during the rise (dP/dt = max) late during the rise (dP/dt = 0 80 max), at the peak and during the decline of the twitch. Muscle length was 6 0 mm. For further details see text.

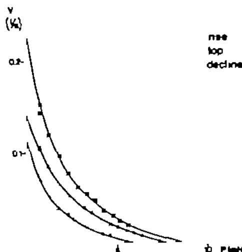


Fig 2 Force–velocity relations late during the rising phase (dP/dt = 0 80 max), at peak and during decline of the twitch. Full lines show the best computed fitts to Hill equation.

after the initial elastic recoil The tension records show that there is some inertial oscillations in force immediately after the release. At 100 ms the initial rapid phase of shortening and the oscillations have subsided This is where shortening velocity was measured With releases at the peak of the twitch and especially on the decline of tension the shortening curve is seen to be very bent.

The early quick release during the rising phase was generally made at the time where dP/dt was maximal. The later quick release in the rising phase was made at dP/dt = 0.80 max. The release during the tension decline was usually made when isometric tension had fallen to about the same value as when the late quick release during the rising phase was made Generally the afterload was increased in small steps and when the highest value was reached the afterload was decreased to values intermediate to the earlier steps. In this way at least 10 force-velocity values were obtained for each release time studied The results were then plotted as in Fig 2 (which for clarity lacks values for the early rising phase) By use of the computer programme the best fittings to Hill's equation were obtained. This is shown by the full lines in Fig. 2. T be noted in this figure is that P at the top of the twitch and the extrapolated P during the rising phase are quite close whereas V_{max} is much higher in the rising phase than at the peak. The force-velocity curve obtained during relaxation resembles the peak curve more than the curve from the rising phase V_{max} and P for the early rising phase was generally somewhat lower than those obtained for the later rising phase. Fig. 3 summarizes the results from all experiments Twitches of different muscles are very similar in their time course as can be seen by the horizontal bars in the figure. Time to peak tension was 2.0±0.1 s (=9) It is clearly seen that, whereas P during the rising phase and at the top of the twitch are fairly equal, V_{max} is much higher in the rising phase compared to the top value. During the decline of the twitch V_{max} and P seem to decrease to about the same extent compared to the values at the top of the twitch

V_{max} was highest during the late rising phase and amounted to 0.304±0 038 muscle lengths per sec

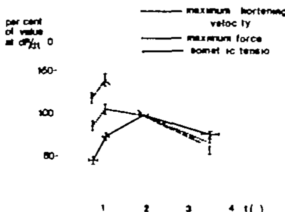


Fig 3 Maximum shortening velocity (V_{max}) extrapolated maximum force (P_0) and isometric tension expressed as percent of their respective values at the peak. Results are given as mean ± S.E. For the peak and the late rise =9 for early rise 5 and for the decline =7 Horizontal bars for the isometric tension values show mean ± S E for the time after the stimulus at which the measurements were made

velocity of the contractile system does not change instantaneously to a new steady level after a load change (see e g Civan & Podolsky 1966) apparently because adjustment of the kinetics in the individual bridges takes a certain time Finally the capacities to shorten and develop tension may be complex functions of the intracellular Ca^{2+} concentration and possibly of the ATP supply to the cross-bridges and need not necessarily have the same time course during a twitch

In spite of the above mentioned objections to the active state concept it is still of interest to see how the ability of a muscle to shorten and develop tension varies during a twitch The present study of force–velocity relations in smooth muscle from rabbit urinary bladder at different stages of reproducible twitches gives information regarding the mechanical output The capacity to shorten and develop tension may however be differently related to the Ca^{2+} transient during the response If the results of studies on multicellular preparations are to be considered relevant for each individual cell it has to be shown that the preparation is homogeneously activated i e that all cells contract and relax at the same time Moreover no part of the muscle must be allowed to shorten excessively in the isometric response thereby stretching other parts Therefore a study regarding the homogeneity of the activation is also included in the present report

METHODS

A total number of 15 rabbits weighing 2.5–3 kg were used The animals were killed by cervical fracture The urinary bladders were emptied dissected out transferred to oxygenated Krebs solution and left in this medium for 1 h at 4°C The bladders were then filled with 10 ml Krebs solution A bundle of longitudinal muscle was marked out, measured and dissected free and finally mounted between two metal clips The length of the strip in situ at 10 ml bladder volume will be referred to as L_{10}. The wet weight of the strips varied between 1 99 and 7 80 mg and the length when mounted between 4 and 6 2 mm corresponding to 0 99±0 07 L_{10} (S E $n=15$)

The recording apparatus (Johansson 1973) was the same as in a previous study (Uvelius 1977) One clip was connected to a force transducer and the other to a light lever (equivalent mass with attached clip 190 mg) The lever could be clamped or released by an electromagnetic device and the load on it could be varied in the range 0–1.5× 10^{-} N by a spiral spring. The compliance of the force transducer was 1 mm/N The lever was undamped. The output of the transducers was recorded on a linear direct writing oscillograph (Devices MX4)

The preparation accommodated for 1 h at 37°C in a physiological salt solution of the following composition in mM NaCl 120 KCl 6.0 $MgCl_2$ 1 $CaCl_2$ 2.5 glucose 11.5 and tris(hydroxymethyl)-aminomethane (Trizma Base Sigma Chemical Co) 23 The solution had been titrated with HCl at 37°C to a pH of 7 4 and was continuously bubbled with 100% O_2. After the accommodation period the temperature was lowered to and held at 25°C and the bathing medium was changed to a tris solution of the above composition but titrated to have pH 7 4 at 25°C. This was also continuously oxygenated. At 25°C almost all spontaneous contractile activity disappeared The muscle was stimulated by single square wave pulses (5 ms 100 V) at a frequency of 0 03–0.06 Hz. The stimulations induced single twitches that remained identical for more than one hour of stimulation A quick release was made on every fourth twitch leaving the other 3 twitches isometric Immediately after the release some inertial oscillations together with a phase of rapid shortening appeared (see further in Johansson Hellstrand & Uvelius 1978) 100 ms after the release both oscillations and the rapid shortening had subsided and this was therefore chosen as the time for measurement of shortening velocity which was determined by drawing the tangent at 100 ms on the shortening curve To measure shortening velocities at a fixed time like this implies that the measurements for different afterloads are not made at the same muscle length. The total length variations including the initial elastic recoil did however not exceed 4.5% of the muscle length

The results were fitted to Hill's equation ($V(P+a) = b(P_0-P)$ Hill 1939) by using a computer programme that took into consideration variations both along the abscissa and the ordinate and weighed all points equal

In the experiments designed to determine the homogeneity of activation the preparation was dipped for a few seconds in a Krebs solution mixed with ground charcoal This marked the preparation with numerous charcoal grains that could not be washed away For the photography a Nikon F2 camera house equipped with a Medical Nikkor lens (200 mm f5 6) and a motor drive was used The camera was started by an impulse from the muscle stimulator Three or 4 exposures per second were made Exposure time was 8 7 ms Exposure time and time between exposures were checked electronically Magnification from preparation to negative was about ×5

In all experiments passive tension was 1 3% of active Cross sectional area was determined from the weight and the length of the preparation assuming a density of 1 mg per mm tissue Results are given below as mean ± standard error unless otherwise stated

RESULTS

A Force–velocity relations

Fig 1 shows typical quick releases to the same afterload early in the rising phase later during the rising phase at the peak and during decline of isometric force For all the releases it can be seen that shortening is very rapid during the first 75–100 ms

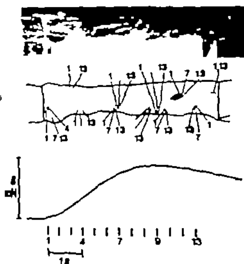


Fig 4 Results from one of the experiments performed in order to determine inhomogeneity of activation. The photograph at the top shows the preparation before stimulation. Muscle length was 5.3 mm. Below the photograph a schematic drawing of the preparation is shown with the movements of the chosen charcoal grains marked out. The numbers refer to the exposures indicated below the registration of the isometric twitch. It is clearly seen that the movement of the grains is very limited.

DISCUSSION

In the present study of twitch contractions of urinary bladder smooth muscle the value for P_0 at the peak was rather low 1.72 N/cm² compared to 12.5 N/cm² in a previous study on KCl activated bladder muscle at 37°C (Uvelius 1976). When the muscles in the present study were tetanized peak tension increased considerably (4–10 times) It is thus very likely that the muscle cells are not maximally activated by a single stimulation. The observations regarding homogeneity of activation presented above indicate that the low contractile force during the twitch is not due to asynchrony between cells

In the present study V_{max} was 0.30 l/s at the rise and 0.22 l/s at the peak compared to 0.29 l/s in the earlier study by Uvelius (1977) If the present experiments were carried out at 37°C and in fully activated muscle it is reasonable to assume that V_{max} would be higher than 0 30 l/s. KCl-activated muscle would thus have V_{max} values lower than those expected for electrically stimulated muscle at the same temperature.

The series elastic element in bladder muscle is more compliant than what is usually found in skeletal muscle (Johansson Hellstrand & Uvelius 1978). The initial elastic recoil in the bladder muscle amounts to about 3% of the muscle length when the preparation is released to shorten against a minimal load In isometrically contracted skeletal muscle a shortening by 0.5% of the central length-controlled section makes tension drop to zero (Ford Huxley & Simmons 1977). The authors attributed this to the elastic behaviour of the crossbridges themselves.

Movement of a certain point on the muscle during an isometric twitch implies that some part of the muscle shortens thereby stretching others In the present study the charcoal grain movement was up to 4% of the muscle length. If the muscle then is released to zero tension it almost momentarily shortens about 3% (the SE recoil) There might be a relation between the SE recoil and the stretching of parts of the preparation during the twitch The photographic recording in the present study is however too slow to make it possible to analyze the immediate recoil after the twitch The elasticity responsible for the initial recoil in the bladder preparation is probably too compliant to reside to a great extent in crossbridges, but the recent studies by Hellstrand & Johansson (1979) and Hellstrand (1979) do suggest that part of the elasticity of this tissue is associated with the contractile units However due to the presence of significant "true series elasticity" it is most likely that the contractile machinery shortens during a twitch even if the muscle length as a whole is held constant. This should mean that the tension output at any moment of the twitch does not correspond to the instantaneous ability of the contractile system to generate isometric force The extrapolation of P_0 from instantaneous force–velocity observations should in that case give a relative estimation of the maximal number of crossbridges that can produce tension at the time studied during the twitch. The quick release method used in the present study does not seem to have any deactivating effect on the contractile machinary of the bladder preparation (Johansson, Hellstrand & Uvelius 1978) The capacity to produce force may however be a complicated function of the level of activation in terms of Ca^{2+} binding to regulatory proteins (Julian & Moss 1976) and the present study provides no information in that respect.

V_{max} may be another function of the level of activation but it cannot be expected a priori that

Table 1 V_{max}, P and the dynamic constants of Hill's equation during the different parts of the twitch, expressed in percent of their respective values at the peak

The absolute values of the parameters at the peak are given in parentheses. The quotients a/P and b/a are calculated from their respective values in each individual experiment and not from the mean values of a, b and P

	Early rise ($n=5$)	Late rise ($n=9$)	Peak ($n=9$)	Decline ($n=7$)
V_{max}	118± 7	141± 8	100 (0 216±0 020 l/s)	67± 7
P	86± 6	107± 7	100 (1 22±0 15 N/cm²)	69± 3
a	94±19	99±17	100 (0 254±0 045 N/cm²)	133±32
a/P	116±31	112±24	100 (0.218±0 036)	196±5
b	141±34	132±24	100 (0 0430±0 0054 l/s)	112±18
b/a	144± 8	132± 6	100 (0 199±0 035 l s cm² N)	98±10

ond whereas it was 0 216±0 020 l/s at the peak of the twitch (for both $n=9$). Table 1 shows the parameters of the force–velocity curves obtained during the twitch in percent of their respective peak values. As the P_s value at the peak (1 22 N/cm²) was considered rather low, two muscles were tetanized by increasing the stimulus frequency to 5 Hz. Tetanus twitch ratio for these were 4 : 1 and 10 : 1

B *Homogeneity of contraction*

Some of the results in the preceding section may be explained by assuming that a considerable inhomogeneity with regard to muscle activation existed, especially at the top and during the decline of the twitch. To see if this was the case a total number of 6 muscles were marked with charcoal grains (see methods) and the following experiments were done

Three muscles were studied during isometric contractions only. Another group of 3 muscles was used for photography of both isometric twitches and quick releases. Fig 4 shows a typical experiment where only isometric tension was recorded. The upper part of the figure shows the preparation marked with charcoal grains. Some of these were chosen as markers to detect tissue inhomogeneity of activity. The movement of these could be followed from picture to picture as indicated in the schematic drawing. It can be seen that the movement of the grains is very limited. The figure actually shows the preparation that had the largest grain movements. For this as well as for the other 5 preparations the greatest movement of a grain never exceeded 4% of the total length of the preparation during isometric contraction. Areas that yielded during the tension decline were not seen in any preparation. Grains close to the clamps did not move more than the rest

To see if inhomogeneity with respect to shortening would appear during isotonic contraction, quick releases to a given afterload (which was about 50% of peak tension) were photographed. The releases were made at $dP/dt=0$ and $dP/dt=$max. Shortening was measured between 140 and 360 ms after release for the distance between the clips and also shortening for a central segment excluding the ends of the strip. This central segment had an isometric length of about 50% of that for the whole preparation. Mean shortening velocity for the period was then calculated. The reason for excluding the period closer to the release was to avoid an influence of the phase with the rapid shortening. For the decline of the twitch no reliable values for the shortening were obtained as they were too small to be detected with any accuracy on the prints. The shortening velocity of the whole muscle divided by the shortening velocity of the middle segment (both expressed as their actual muscle lengths per second) was 0 91±0 28 (S D , $n=3$) for the rise and 0 91±0 19 (S D , $n=3$) for the top of the twitch. (If all parts of the muscle shortened with the same velocity the quotient should be 1.)

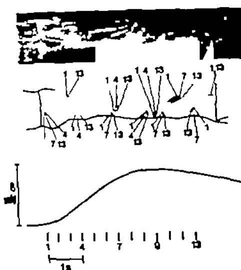


Fig. 4. Results from one of the experiments performed in order to determine inhomogeneity of activation. The photograph at the top shows the preparation before stimulation. Muscle length was 5 3 mm. Below the photograph a schematic drawing of the preparation is shown with the movements of the chosen charcoal grains marked out. The numbers refer to the exposures indicated below the registration of the isometric twitch. It is clearly seen that the movement of the grains is very limited.

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V_{max} may be another function of the level of activation, but it cannot be expected a priori that

V_{max} should have the same time dependence during the twitch as P_0. Moreover if an internal load exists the extrapolated V_{max} does not represent shortening velocity of the contractile machinery against zero load Siegman et al (1976) have suggested that attached crossbridges exist in resting smooth muscle If such bridges in "rigor state" would exist also in the active muscle they could contribute to an internal load and this factor may vary with time in the course of the twitch Since this limitation on shortening velocity cannot be excluded in the present experiments it is not possible to say if V_{max} is more closely related than P to the level of activation in this preparation

The results show that there is a greater difference between V_{max} values during the rising phase of the twitch and at the peak than between the corresponding P_0 values This could be due to inhomogeneity of activation as suggested to occur in spontaneous phasic contractions of rat portal vein (Hellstrand & Johansson 1975) The photographs of the marked strips in the present study indicate however that the electrically stimulated bladder muscle is very homogeneously contracting During an isometric twitch the greatest movement of a charcoal grain on the muscle surface never exceeded 4% of the muscle length This is comparable to marker movements of up to 5% for KCl activated strips of pig carotid artery (Driska Damon & Murphy 1978) The present experiments also showed that the regions of the strip close to the clamps did not yield during the twitch A previous study (Uvelius 1976) showed that the smooth muscle cells in urinary bladder preparation are coupled in series and that a length change of the muscle is accompanied by the same relative length change of the individual muscle cell The different time dependence of V_{max} and P_0 in the present study therefore appears to be applicable to the individual cell It is possibly a characteristic of the contractile machinery itself

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Acta Physiol Scand 1979 106: 487–488

The effect of exercise on high density (HDL) lipoprotein apoproteins

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Key words apoprotein cholesterol high density lipoproteins, serum; physical activity

Epidemiological studies have indicated that premature coronary heart disease (Nikkilä 1953 Carlson & Ericsson 1975) and ischemic cerebrovascular disease (Rössner et al. 1978) are associated with low levels of HDL cholesterol It has been suggested that a high level of HDL may protect against vascular disease (Glueck et al 1977) The pathophysiological mechanism underlying these observations is not known. One suggestion is that HDL transports cholesterol from the periphery to the liver and thereby decreases lipid accumulation in the vascular wall. In most studies cholesterol has been used as an indicator for HDL. However a change in HDL cholesterol may reflect only a change in the proportion of cholesterol in relation to the other lipoprotein components i.e the apoproteins, A I and A-II and phospholipids. As physical activity seems to cause higher HDL cholesterol levels (Wood et al 1976, Lehtonen & Viikari 1978) it was of interest to study whether this increase could also be demonstrated for the apoproteins A I and A II or whether it was a result of an increased cholesterol protein ratio We now report both on the amounts of apoproteins A-I and A-II and on the HDL cholesterol concentrations in men undergoing regular physical training.

Material and methods

Fasting serum was studied from 23 athletes and 15 age matched controls. The athletes exercised 4 or more times weekly and as an average run at least 25 km per week The radial immunodiffusion (RID) procedure for the quantitation of A-I and A II was similar to that described by Cheung & Albers 1977 The HDL cholesterol was measured as described by Viikari 1976.

Results

The athletes had significantly higher apolipoprotein A I and HDL cholesterol concentrations than the controls (Table 1) The highest concentrations of HDL cholesterol were found in the athletes who had exercised most (run >70 km per week) No significant difference was noted in apolipoprotein A II concentration between the groups.

In about half of the athletes (11/23) apolipoprotein A I concentrations were above the upper limit of the controls (determined as the mean ±2 S D). In the control group HDL cholesterol concentrations showed a significant correlation with apolipoprotein A I ($P<0.001$) but not with apolipoprotein A II concentrations No correlation

Table 1 *HDL in athletes and controls (mean ±S.D)*

HDL high-density lipoprotein, Apo A-I = apoprotein A-I, Apo A-II apoprotein A-II

	Athletes (=23)		Control (=15)
HDL cholesterol (mmol/l)	1 77±0.39	$P<0.01$	1 42±0.31
Apoprotein A-I (g/l)	2 16±0.29	$P<0.001$	1 65±0 28
Apoprotein A-II (g/l)	0.55±0 09	NS	0 49+0 11
Apo A-I Apo A-II	3 98±0 59	$P<0.01$	3 45±0.53
HDL cholesterol/Apo A-I	0.83±0 18	NS	0.89±0 10
HDL cholesterol/Apo A-II	3.28±0 83	NS	2.95±0 59

V_m should have the same time dependence during the twitch as P_0. Moreover if an internal load exists the extrapolated V_{max} does not represent shortening velocity of the contractile machinery against zero load Siegman et al (1976) have suggested that attached crossbridges exist in resting smooth muscle If such bridges in "rigor state" would exist also in the active muscle they could contribute to an internal load and this factor may vary with time in the course of the twitch Since this limitation on shortening velocity cannot be excluded in the present experiments it is not possible to say if V_{max} is more closely related than P_0 to the level of activation in this preparation

The results show that there is a greater difference between V_{max} values during the rising phase of the twitch and at the peak than between the corresponding P values This could be due to inhomogeneity of activation as suggested to occur in spontaneous phasic contractions of rat portal vein (Hellstrand & Johansson 1975) The photographs of the marked strips in the present study indicate however that the electrically stimulated bladder muscle is very homogeneously contracting During an isometric twitch the greatest movement of a charcoal grain on the muscle surface never exceeded 4% of the muscle length This is comparable to marker movements of up to 5% for KCl activated strips of pig carotid artery (Driska Damon & Murphy 1978) The present experiments also showed that the regions of the strip close to the clamps did not yield during the twitch A previous study (Uvelius 1976) showed that the smooth muscle cells in urinary bladder preparation are coupled in series and that a length change of the muscle is accompanied by the same relative length change of the individual muscle cell The different time dependence of V_{max} and P_0 in the present study therefore appears to be applicable to the individual cell It is possibly a characteristic of the contractile machinery itself

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The effect of exercise on high density (HDL) lipoprotein apoproteins

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Key words apoprotein, cholesterol high density lipoprotein; serum; physical activity

Epidemiological studies have indicated that premature coronary heart disease (Nikkilä 1953 Carlson & Ericsson 1975) and ischemic cerebrovascular disease (Rössner et al. 1978) are associated with low levels of HDL cholesterol It has been suggested that a high level of HDL may protect against vascular disease (Glueck et al 1977) The pathophysiological mechanism underlying these observations is not known One suggestion is that HDL transports cholesterol from the periphery to the liver and thereby decreases lipid accumulation in the vascular wall In most studies cholesterol has been used as an indicator for HDL. However a change in HDL cholesterol may reflect only a change in the proportion of cholesterol in relation to the other lipoprotein components i e the apoproteins A I and A-II and phospholipids. As physical activity seems to cause higher HDL cholesterol levels (Wood et al., 1976, Lehtonen & Viikari 1978) it was of interest to study whether this increase could also be demonstrated for the apoproteins A-I and A II or whether it was result of an increased cholesterol protein ratio We now report both on the amounts of apoproteins A-I and A-II and on the HDL cholesterol concentrations in men undergoing regular physical training.

Material and methods

Fasting serum was studied from 23 athletes and 15 age matched controls The athletes exercised 4 or more times weekly and as an average run at least 35 km per week The radial immunodiffusion (RID) procedure for the quantitation of A I and A II was similar to that described by Cheung & Albers 1977 The HDL cholesterol was measured as described by Viikari 1976.

Results

The athletes had significantly higher apolipoprotein A I and HDL cholesterol concentrations than the controls (Table 1) The highest concentrations of HDL cholesterol were found in the athletes, who had exercised most (run >70 km per week) No significant difference was noted in apolipoprotein A II concentration between the groups.

In about half of the athletes (11/23) apolipoprotein A I concentrations were above the upper limit of the controls (determined as the mean ±2 S D) In the control group HDL cholesterol concentrations showed a significant correlation with apolipoprotein A I ($P<0.001$) but not with apolipoprotein A II concentrations. No correlation

Table 1 *HDL in athletes and controls (mean ±S.D)*

HDL high-density lipoprotein Apo A-I apoprotein A-I Apo A-II apoprotein A-II

	Athletes (n=23)		Controls (n=15)
HDL cholesterol (mmol/l)	1 77±0.39	$P<0.01$	1 42±0.31
Apoprotein A-I (g/l)	2 16±0 29	$P<0.001$	1 65±0.28
Apoprotein A-II (g/l)	0.55±0 09	NS	0 49±0 11
Apo A-I/Apo A-II	3.98±0.59	$P<0.01$	3 45±0.53
HDL cholesterol/Apo A-I	0 83±0.18	NS	0 89±0 10
HDL cholesterol/Apo A-II	3 28±0 83	NS	2 95±0 59

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Defective ion transport in diabetic mouse islet cells

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Because D-glucose inhibited Rb efflux from pancreatic islets, it was suggested that depolarization of β-cells and insulin release are in part regulated by K electrodiffusion (Sehlin & Täljedal 1975) The results and the hypothesis are supported by subsequent radio-tracer studies and electrophysiological recordings in other systems (Henquin 1978 Boschero & Meissner 1978 Malaisse et al. 1978 Atwater et al. 1978). Thus the importance of K permeability changes in mediating the normal insulin-releasing action of D-glucose appears to gain in acceptance. We report here that the hypothesis can help to explain the defective control of insulin secretion that occurs in a strain of severely diabetic mice.

C57BL/KsJ mice of both sexes were taken from a local colony (established by breeding couples from the Jackson Laboratories, Bar Harbor Maine USA) and starved overnight. Animals homozygous for the gene, *db* were selected for study when a constant fall of their body weight for 2–4 weeks indicated transition from the phase of mild hyperglycemia to that of severe insulin-deficient diabetes (Berglund et al 1978) Their age ranged 27–36 weeks with a median of 28 weeks. Similarly aged and sex-matched non-diabetic controls lacking the *db* gene were obtained from the same inbred strain. Collagenase-isolated islets of Langerhans were labelled with ⁸⁶RbCl briefly washed (approx 1 min) in non-radioactive medium and subsequently transferred to a micro-perifusion system allowing detailed measurements of the rate of Rb efflux. The basal medium used for labelling with ⁸⁶Rb and for subsequent perifusion was Krebs-Ringer bicarbonate (De Luca & Cohen 1964), except that the bicarbonate was replaced by 20 mM Hepes (*N* 2 hydroxyethylpiperazine-*N'* yl-ethane-sulfonic acid), pH 7.4 All media had a temperature of 37°C contained 0.5% (w/) bovine serum albumin, and were equilibrated with ambient air The labelling medium contained 3 mM D-glucose and 28 μM ⁸⁶RbCl (71 TBq/mol) The perifusion medium contained D-glucose as indicated

Fig. 1 shows the time course of Rb efflux from islets perifused with 3 mM D-glucose for 10 min and then suddenly exposed to 20 mM D-glucose The efflux did not strictly follow first-order kinetics. However to facilitate comparison of the curves over limited incubation periods, they can still be approximated by straight lines in semi-logarithmic plots. Such analyses showed that the basal rate constant for Rb release in the presence of 3 mM D-glucose was significantly smaller in diabetic mice than in normal controls. Mean values ±S E. for the approximate slopes of lines in min 3.5–9.5 were -0.045 ± 0.002 for controls and -0.030 ± 0.002 for diabetic mice (*t*-test. $P<0.001$ Wilcoxon's rank

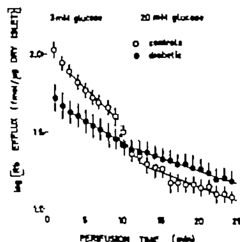


Fig 1 Efflux of Rb with time from pancreatic islets of normal C57BL/KsJ mice (O) and severely diabetic C57BL/KsJ-*db/db* mice (●) The logarithm (base 10) of Rb in the effluent (fmol/μg dry weight of islets) is shown on the ordinate and presented as mean values ±S.E. for 7 different expts. Solid lines between min 3.5 and 9.5 and between min 13.5 and 24.5 were calculated by the method of least squares. Broken lines between min 9.5 and 13.5 were drawn free-hand

between A I and HDL cholesterol could be demonstrated in the athletic group

The composition of HDL in the athletes did not differ significantly from that of the control HDL as judged from the ratio of HDL cholesterol to A I and A-II

Comment

Our results confirm previous studies which demonstrate that persons with high physical activity in leisure time have higher HDL cholesterol levels than physically inactive persons This study also shows that this increase in HDL cholesterol is accompanied by a rise in apoprotein A I concentration Thus at least under these physiological conditions the cholesterol to protein ratio in HDL is not significantly changed In this study the amount of apoprotein A II increases slightly but not significantly causing a significant change in the ratio of A I to A II One function of apoprotein A I is the activation of LCAT (lecithin cholesterol acyltransferase) enzyme which is responsible for the formation of most of cholesteryl esters in the plasma (Fielding et al 1972 Soutar et al 1975) It has been suggested that one major role of LCAT is to promote the transfer of cholesterol from peripheral tissues to the liver (Glomset & Norum 1973) Physical exercise thus increases both HDL cholesterol and apolipoprotein A I both of which can be considered as beneficial metabolic effects

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Urea-induced inhibition of antidiuretic hormone (ADH) secretion

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The relative importance of juxtaventricular sodium sensitive receptors vs. osmoreceptors (Verney 1947) in the cerebral control of water balance remains controversial (cf Andersson 1978) Among the supports of the former mechanism is the observation that lowering of the cerebrospinal fluid (CSF) [Na] by intraventricular infusions of pure saccharide and urea solutions inhibit the basic ADH-secretion in non-hydrated goats (Eriksson 1974) However urea also plays a role in the argumentation for cerebral osmoreceptors Intracarotid infusions of hypertonic urea have been shown to elevate the CSF [Na] of sheep to the same extent as corresponding infusions of hypertonic NaCl and sucrose, although (in contrast to NaCl and sucrose) intracarotid urea has only weak antidiuretic and dipsogenic effects. Together with observations favouring juxtaventricular sodium sensitivity in the sheep this has given rise to the suggestion that a dual osmoreceptor–sodium sensor system may participate in regulating ADH-secretion and thirst. The osmoreceptor part of the system would then be located in a brain region devoid of a blood-brain barrier (BBB), where blood borne urea (due to its high permeation into cells) would not act as an efficient osmotic stimulus (McKinley Denton & Weisinger 1978) An alternative explanation could be that urea reduces the activity of juxtaventricular sodium sensitive receptors Therefore it was of interest to compare the effects on water balance of IVT infusions of urea and two other cell membrane permeating substances (ethanol and acetone) administered in 0.15 M NaCl. Four female goats (b wt. 30 to 35 kg) were used. The animals were supplied with permanent platinum-iridium cannulas (Åkerlund, Andersson & Olsson 1973) in the lateral ventricle where infusions were made for 1 h periods at a *rate of 20 µl/min.* Urine was collected in 10 min samples Samples of CSF (0 3 ml) for Na and K determinations were taken via the IVT cannula before and at intervals after the infusions. Solutions administered into the lateral ventricle were 0.15 M urea (=3), ethanol (n=3) and acetone (=3) dissolved in 0 15 M NaCl This provided an osmolality of the solutions of 0 45 Osm/kg

As shown in Fig. 1 the IVT administration of urea in 0.15 M NaCl invariably induced positive renal free water clearance (C_{H_2O}) after about 30 min in the non-hydrated goats. The water diuresis outlasted the infusion by about 30 min. Five after termination of the infusion the [Na] and [K] in the CSF of the lateral ventricle were reduced from pre infusion levels by 4 7±0 4% and approximately 10% respectively These concentrations had again reached pre-infusion levels when the renal C_{H_2O} returned to a negative value Neither the infusions of ethanol and acetone in 0 15 M NaCl nor control infusions of merely 0.15 M NaCl (n=4) induced any water diuresis or obvious *fall in the CSF* [Na]. However the reduction *of CSF* [K] *5 min after the* ethanol and acetone infusions was of the same order as in the urea expts.

Discussion Although sodium was added to the infusions in normal CSF concentration in the present experiments, the IVT administration of urea effectively lowered the CSF [Na] and inhibited the basic ADH-secretion of the non-hydrated goats (=elicited a water diuresis) Identical effects were previously reported in the same species as regards IVT infusions of glycerol dissolved in 0 16 M NaCl (Olsson Larsson & Liljekvist 1976) A well developed BBB exists for as well urea as for glycerol (cf Rapoport 1976) Therefore the efficiency of IVT urea and glycerol to lower the CSF [Na] might be due to osmotic transport of water from the blood to the CSF The lack of effect of ethanol and acetone which easily pass over the BBB is in agreement with this assumption. That a water diuresis developed concomitant with the urea-induced fall in CSF [Na] could be regarded as further support for the existence of juxtaventricular sodium sensitive receptors. However since urea passes rapidly over plasma membranes into cells, an osmotic drag of water from the blood may also have induced cell volume expansion inside the BBB

sum test $P<0.01$) In agreement with our findings for the well-functioning islets of non-inbred *ob/ob*-mice (Sehlin & Täljedal 1975) the normal C57BL/KsJ mouse islets responded to 20 mM D-glucose with a sudden fall of the Rb^+ efflux rate This effect of raising the glucose concentration was not seen in the diabetic mouse islets The slopes in min 13 5–24 5 were -0.019 ± 0.007 (controls) and -0.016 ± 0.002 (diabetic)

The results strongly suggest that the basal Rb permeability in the β-cells of KsJ-*db/db* mice is abnormally low That this is the case has not previously been shown but conforms with measurements of Rb retention and accumulation by islets in static incubations (Berglund et al 1978) On the reasonable assumption that Rb^+ is a functional analogue of K^+ (Sehlin & Täljedal 1975 Henquin & Meissner 1978) the findings also support the hypothesis that insulin relase is normally dependent on a glucose-sensitive mechanism for controlling K^+ permeability in the β-cells Moreover they demonstrate that this mechanism is defective in at least one form of severe diabetes mellitus In comparison with normal controls the diabetic mouse β-cells exhibit a smaller membrane electric potential under basal conditions (Meissner & Schmidt 1976) a higher basal release of insulin from the perfused pancreas (Berglund et al 1978) and loss of the electric (Meissner & Schmidt 1976) and secretory (Berglund et al 1978 Boquist et al 1974) responses to high glucose concentrations These abnormalities are logical implications of the original hypothesis (Sehlin & Täljedal 1975) conjoined with the present data

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Insensitivity to insulin of human arterial tissue in vitro

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The local metabolism of the vascular wall is probably of importance for the development of vascular diseases (Ross & Glomset 1977). Both insulin deficiency and insulin excess have been suggested as causative factors of vascular disease (Stout 1977*a*). Whether insulin directly influences the metabolism of human arterial tissue is not known. In intima-media preparations of animal arteries, containing a high proportion of smooth muscle, moderate effects of insulin on glucose metabolism, amino acid transport, and incorporation of amino acid into protein are found during in vitro incubations for a few hours (for review see Arnqvist 1977). The effects of insulin on vascular smooth muscle in vitro can only be demonstrated at high unphysiological insulin concentrations (≥0 001 U/ml).

We have investigated the effect of insulin (0.1 U/ml) on the metabolism of human renal arteries in vitro. Pieces of renal arteries were obtained from kidneys removed at nephrectomy. The cause of nephrectomy was in most cases renal carcinoma. The mean age of the patients was 56.2 (range 29–77 years). Altogether renal artery samples were obtained from 20 patients. None of the patients had diabetes mellitus. Immediately after excision of the kidney a piece of the renal artery was removed, laid in isotonic saline at room temperature and brought to the laboratory within a couple of minutes. The renal artery was carefully dissected free from perivascular tissue and the adventitia was removed as far as possible. Arteries with visible atherosclerotic lesions were discarded. Histological control of the dissection procedure showed that the vascular preparation consisted of intima-media and some adventitia with no perivascular tissue present. The length of the arteries varied from 1 to 3 cm. The arteries were cut up longitudinally and divided into pieces weighing about 50 mg (range 20–100 mg). Adjacent pieces were used as test and control preparations. The effect of insulin (0.1 U/ml) on accumulation of glucose carbon from ^{14}C-labelled glucose, the distribution of the nonutilizable amino acid α-amino-isobutyric acid (AIB) and incorporation of leucine-^{14}C into protein was studied. The arterial samples were incubated in Krebs Henseleit bicarbonate buffer. The methods used for incubation, determination of glucose-^{14}C accumulation and α-amino-isobutyric acid distribution were the same as previously described (Arnqvist 1974). The accumulation of radioactivity from ^{14}C-labelled glucose represent both glucose and glucose metabolites, the major glucose metabolites being lactate and glycogen (Arnqvist 1973). Incorporation of leucine-^{14}C into protein was analysed according to Arnqvist and Dahlkvist (1979).

Insulin had no significant effect on the accumulation of glucose-^{14}C carbon or the distribution of AIB during incubation times of 60–180 min, nor did insulin affect the incorporation of leucine-^{14}C into protein during incubation for 360 min (Table 1). The distribution of AIB calculated as per cent of the wet tissue weight exceeded 100% which suggests that AIB in renal arteries, as in other vascular preparations (Arnqvist 1977), was accumulated intracellularly by an active transport system.

The present results suggest that the metabolism of human arterial tissue is insensitive to insulin with regard to acute (≤3 h) effects on glucose metabolism, amino acid transport and incorporation of amino acid into protein. It is conceivable that insulin effects may have been elicited by even higher insulin concentrations, but since the concentration used (0 1 U/ml) was already far above the physiological range (10–100 μU/ml) it was not considered of interest to try higher hormone concentrations. Smooth muscle cells constitutes the bulk of the cellular mass of the intima-media preparation and it is reasonable to assume that they make the preponderant contribution to the composite metabolic activities of the preparation. The single

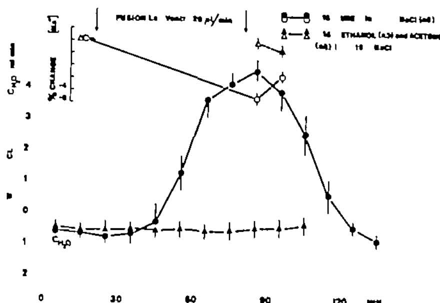


Fig 1 Reduction of CSF Na and inhibition of the basic ADH secretion (=induction of a water diuresis) in the non-hydrated goat elicited by the IVT infusion of 0 15 M urea in isotonic (0 15 M) NaCl (open and filled circles) For comparison is shown the absence of such effects in response to the corresponding infusion of ethanol or acetone in 0 15 M NaCl (open and filled triangles)

(=inhibition of tentative osmoreceptors) Hence the present study appears inconclusive as regards the relative importance of juxtaventricular sodium sensitivity vs cerebral osmoreceptors

Glycerol inhibits Na-K ATPase activity (Mayer & Avi Dor 1970) and it has been suggested that reduced trans-choroidal Na transport may contribute to the lowering of the CSF [Na] induced by IVT glycerol (Olsson et al 1976) Inhibition of ADH secretion and thirst has been induced also by the IVT administration of some other known inhibitors of Na K ATPase which hints at the possibility that enzymatic cation transport may be essential for the excitation of cerebral receptors involved in the control of water balance (cf Andersson 1978) Among substances shown to inhibit Na-K ATPase activity is also urea (Skou & Hilberg 1965) Thus it cannot be excluded that the water diuresis observed in the present study might have been the manifestation of urea-induced inhibition exerted at the receptor level If this is the reason why cerebral dehydration and elevated CSF Na induced by intracarotid urea is a poor stimulus of ADH release and thirst, it appears likely that the topical receptors are directly accessible both to CSF and blood borne influences

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Vanadate-induced oliguria in the anesthetized cat

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Key words. Vanadate, oliguria, vasoconstriction, renal blood flow

Considerable interest has been focused on vanadate since its discovery as a very efficient inhibitor of Na,K-ATPase (Josephson & Cantley 1977 Cantley et al. 1977) It was reported recently that vanadate acts as a most potent diuretic in rats possibly in part as a result of an inhibition of sodium reabsorption in the kidney tubules (Balfour et al 1978) The present experiments demonstrate that in the anesthetized cat vanadate causes severe vasoconstriction of the renal blood vessels accompanied by an immediate, marked and reversible reduction in urine production.

The cats were fasted overnight with free access to water and anesthetized with chloralose Catheters were placed in a femoral artery and in veins for blood pressure measurements blood sampling and infusion. Urine was collected in 10 min periods via a catheter in the urinary bladder or ureters. In some experiments blood flow in the renal artery was recorded continuously by means of an electromagnetic flowmeter (Nycotron, Oslo) Vanadate (sodium orthovanadate) was dissolved in isotonic saline and given i.v either as a single dose (1 ml in 1 min) or continuously (0 1 ml min) The results are given as mean values ± S E. and like vanadate doses they are expressed in relation to body weight (kg)

When given as a single dose (5 μmol kg) comparable to that used in rats (Balfour et al 1978) vanadate caused an immediate fall in urine flow from 7 94±0 65 μl kg min to 2.67±0.83 μl kg min followed by a slow increase to a peak value of 9 30±0 74 μl kg min ($n=4$) When vanadate was given as a continuous infusion no effect on diuresis was observed at dose rates of up to 0.1 μmol kg min However infusion of 0.5 μmol kg min was constantly accompanied by a marked decrease in urine production associated with a fall in renal blood flow and a rise in arterial blood pressure (Fig. 1) When the infusion was stopped urine flow rose and was parallelled by opposite changes in the above-mentioned parameters. In 4 expts. vanadate caused a fall in renal arterial conductance (calculated as flow divided by

Table 1 *Urine production (μl kg min) and urinary excretion of Na and K (μEq kg min) in the control period and after infusion of sodium orthovanadate (0.5 μmol kg min) when urine flow was maximal*

Only urine production was followed during the infusion period. The post infusion values were not significantly different from the control values

Exp.	Control period			Infusion period (urine flow)	Post infusion period (at peak flow)		
	Urine flow	Na	K		Urine flow	Na	K
1	8.37	1.16	1.65	0.77	9.90	0.65	1.73
2	9.00	1.34	1.37	0.24	13.57	1.79	2.04
3	4.18	0.81	0.98	0.52	28.13	4.27	7.77
4	5.92	0.54	0.68	0.26	6.22	0.29	1.06
5	11.26	0.68	5.44	1.85	27.27	4.20	7.33
6	8.82	0.22	0.85	0.01	11.60	0.27	1.14
Mean S E.	7.93±1.02	0.79±0.17	1.82±0.74	0.61±0.27	15.94±3.70	1.91±0.76	3.51±1.28

Table 1 *Effect of insulin on accumulation of glucose-^{14}C distribution of α-aminoisobutyric acid-^{14}C and incorporation of leucine ^{14}C int protein in intima-media of human renal arteries (mean ± S.E)*

Metabolic parameter	Incubation time (min)	Number	Control	Insulin (0 1 U/ml)
Accumulation of	60	7	87±4	90±5
glucose ^{14}C carbon	120	5	94±5	91±3
(μmol/g w w)	180	7	98±3	101±3
Distribution	60	5	89±5	94±2
α-aminoisobutyric acid ^{14}C	120	4	109±11	111±9
(% of wet weight)	180	5	140±13	144±6
Incorporation of leucine ^{14}C into protein (DPM/mg protein)	360	7	1 19?±.56	1 212±231

layer of endothelial cells represents only a small fraction of the cellular mass of the intima-media and whether these cells are sensitive to insulin can therefore not be deduced from the results obtained In bovine mesenteric artery glucose-^{14}C accumula tion AIB transport and incorporation of amino acid into protein were moderately stimulated by high concentrations of insulin (Arnqvist 1974) while no effects were found on these metabolic processes in the human renal arteries The difference in insulin effects between the two intima-media preparations may be due to species differences since the sen sitivity to insulin varies with species (Di Girolamo & Rudman 1966)

In diabetic rabbit aorta the glucose metabolism is impaired and is stimulated by insulin treatment in vivo for 42 h but not earlier (Mulcahy & Winegrad 1962) and in diabetic rat aorta the incorporation of amino acid into protein is reduced and is stimulated only after insulin treatment for 48 h (Arnqvist & Dahlkvist 1979) In studies in vitro with tissue cul ture technique effects of insulin in physiological concentrations on synthetic processes and cell proliferation in vascular smooth muscle of animal origin have been reported by some authors (Stout Bierman & Ross 1975 Stout 1977b) but not by others (Ledet 1976, Arnqvist & Norrby 1979) To find out whether insulin has any direct effects on biosynthetic processes and cell proliferation in hu man vascular smooth muscle which appear after several hours to days experiments with tissue cul ture technique have to be done

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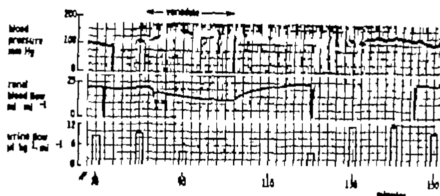


Fig 1 The effect of vanadate (0.5 μmol kg min) on mean arterial blood pressure renal blood flow and urine flow (The rapid deflections in the upper tracings indicate test procedures.)

mean arterial pressure) from 0 058±0 011 to 0 016±0 003 ml min⁻¹ kg mmHg⁻¹ ($0\ 01<P<0\ 02$) Further results from 6 expts in which the infusion period varied from 20–60 min are listed in Table 1 In the post infusion period urine flow exceeded control flow for a short period of time and only peak flows are listed in the table In a few expts the serum concentration of vanadate was determined by a method which takes advantage of the shift in equilibrium binding of (^{48}V) vanadate to Na K ATPase upon addition of an aliquot of serum (Hansen et al 1979) The changes in renal blood flow and blood pressure were evoked at serum vanadate concentrations of about 30 μM

It is realized that both the anesthesia and the operative procedures may influence kidney function and thus interfere with a possible action of vanadate on tubular function However furosemide a diuretic which inhibits tubular reabsorption of Na and Cl was fully active during the present experimental conditions This effect was inhibited by simultaneous infusion of vanadate It may also be that the action of vanadate on vascular smooth muscle is modified by the experimental conditions but alfa or beta-blockers did not change the vascular effect of vanadate It should also be mentioned that by comparing urine flow from each ureter it was found that application of a flowmeter on one renal artery (which includes partial denervation) did not affect control urine flow or the response to vanadate Finally it was found that chloralose or its metabolites did not affect (^{48}V)-vanadate binding to Na K ATPase from ox brain

In conclusion the present results demonstrate that contrary to its effect on kidney function in the rat vanadate causes a marked decrease in urine production in the anesthetized cat This effect appears to be due to a direct constrictive action on the renal blood vessels leading to a fall in glomerular capillary pressure An antidiuretic action of this genesis may obscure the diuretic effect of a possible inhibition of tubular Na,K ATPase and of tubular reabsorption of sodium and water

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